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The effects of pre-inoculation drought stress on the components of partial resistance of rice (*Oryza sativa* L.) to leaf blast (*Magnaporthe grisea* [Herbert] Barr)

by

Henry William Klein-Gebbinck ©

A Thesis Submitted to the Faculty of Graduate Studies and Research in Partial Fulfillment
of the Requirements for the Degree of Doctor of Philosophy

in

Plant Pathology

Department of Agricultural, Food and Nutrition Sciences

Edmonton, Alberta

Fall, 1995

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **The effects of pre-inoculation drought stress on the components of partial resistance of rice (*Oryza sativa* L.) to leaf blast (*Magnaporthe grisea* [Herbert] Barr)** submitted by **Henry William Klein-Gebbinck** in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology.

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Abstract

Blast, caused by *Magnaporthe grisea* Herbert (Barr), is an important disease on *Oryza sativa* L. especially in upland rice culture where drought can alter the susceptibility of the plants to infection.

Pre-inoculation drought stress increased the relative infection efficiency of both susceptible- and resistant-type lesions. Ranking of cultivar partial resistance to blast infection was altered by drought stress. There was a stress threshold above which there was a dramatic increase in the relative infection efficiency. The age of the oldest leaf segment with at least one susceptible lesion was increased by drought stress. The distribution of lesions over different aged segments varied with lesion type. The maximum number of susceptible lesions occurred on 1-2 day younger leaf segments than resistant lesions. Susceptible-type lesion number decreased rapidly whereas resistant-type lesion number decreased slowly with increasing leaf segment age. The rate at which susceptible, but not resistant, lesion numbers decreased with leaf segment age depended on drought stress. The effect of drought stress was observed at all stages of vegetative development, although relative infection efficiency decreased with plant age.

In a simulation study, the effect of prolonging the susceptibility duration of a plant part was effective in increasing the rate of disease progress. The effect was more important for simulated pathosystems in which the host had a short initial susceptibility duration. The effect of increasing susceptiblity duration was similiar regardless of plant growth rates. The effect of increasing the susceptibility duration was as important as an equivalent proportional increase in the infection efficiency or maximum lesion size or decrease in the latent period. At low susceptibility durations, the effect of changes in susceptility duration on disease progress varied with plant-age resistance.

The effect of drought stress was mediated through an increase in both the asymptotic number of infections and the speed at which this asymptote was attained with increasing leaf wetness duration. This effect of stress on increasing the speed of

infection was more important on older than younger leaves. Pre-inoculation drought stress had little or no effect on other epidemiological parameters. Latent period was significantly shorter on stressed plants than unstressed plants but the mean difference did not exceed 5 hours. There was no effect of pre-inoculation drought stress on either lesion size or sporulation.

Foilar infections reduced plant growth and affected root physiology as determined by reductions in both proton extrusion and oxidation of α-naphthylamine. The reduction in shoot and root growth depended on the leaf development stage of the expanding leaf at inoculation. Plant growth was reduced more by foliar infection than mild post-inoculation drought stress. Root growth was stimulated in the deeper soil horizons by mild drought stress. However, this stimulatory effect was negated by disease on the foliage.

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Chapter 1 Literature Review

1.1 Introduction to Rice

Rice (*Oryza sativa* L.), a graminiceous crop, is a major source of food for more than 40% of the world's population. On a global scale, rice accounted for 21% of the total calorie supply in 1986-1988 (IRRI 1991c). In Asia, rice provided an average of 36% of the total calorie supply (8% in Afghanistan and Pakistan to 69% or more in Bengladesh, Laos, and Myanmar). Rice accounted for 12% of the calories consumed in South America (2% in Argentina to 16% in Brazil and 39% in Guyana), 3% in North and Central America (2% in the United States to 21% in the Dominican Republic), 6% in Africa (4% in Zaire to 55% in Madagascar), 1% in Europe, 2% in the Commonwealth of Independent States, and 4% in the Oceania region (IRRI 1991c). Although rice ranks second to wheat in area sown to graminiceous crops, a hectare of rice can sustain 5.7 persons/year compared to 4.1 persons/year for a hectare of wheat (De Datta 1981).

1.2 Botanical Description

Rice belongs to the grass tribe Oryzeae, which is characterized by laterally compressed one-flowered spikelets, and two short glumes (Yoshida 1985). These perfect flowers are arranged in a loose terminal panicle with each panicle bearing a number of spikelets.

An important tissue in rice is the aerenchyma (Yoshida 1985). It consists of cells arranged in a circular fashion to produce tubes which conduct air from the leaves and stems to the root, thereby allowing the plant to survive in waterlogged soils.

1.3 Types of rice and distribution

There are six varietal groups of rice based on isozyme variation (Glaszmann 1986, 1988). The two main groups are the indica (Group I) and japonica (Group VI) varieties, comprising 86% of the rice varieties. Cultivated rice is largely comprised of these groups. Analysis of isozyme patterns showed that Groups II-V were frequently found in the Indian subcontinent, especially along the Himalayan foothills. In contrast, indica rices are evenly distributed in tropical Asia. Genetic diversity in the japonica rices was highest in the hilly areas of southeast Asia, thus indicating that this group probably originated here. Japonica rices are better adapted to the subtropics and temperate zones whereas indica rices are widely grown in the tropics (De Datta 1981).

1.4 Rice ecosystems

Rice is produced in a number of environments including irrigated lowland, rainfed lowland, deepwater and rainfed upland environments (De Datta 1981). In lowland rice culture, bunds or dikes are constructed to collect water. Five to 50 cm of standing water are usually required (De Datta 1981). In the irrigated lowland ecosystems, tranplanting is the most important method of crop establishment, although direct seeding onto puddled soils is also practised in spite of increased risk of lodging (De Datta 1981). Crops are heavily fertilized. Irrigated lowland rice accounted for 57.2% of the total rice production area of the major rice-producing countries in Asia and 25% of the total area of major rice-producing areas in Latin America (IRRI 1991c).

The methods of rice culture are similar in rainfed lowland ecosystems (De Datta 1981). However, seedling establishment, seedbed preparation and transplanting are determined by local rainfall patterns whereas there is no restriction in timing in irrigated lowland rice culture. Alternate periods of flooding and dry conditions are common. Yields vary depending on rainfall and fertilizer use. In Asia and Latin America, rainfed lowland ecosystems accounted for 28.1% and 5.4%, respectively, of the total area of the major rice-producing countries in 1987.

Deepwater rice culture is practised in areas where more than 50 cm of standing water may accumulate for a certain period of time (De Datta 1981). Maximum water depths vary between 51 and 100 cm of water for more than half of the crop duration and sometimes the crop may be completely submerged. The crop is established by direct seeding or transplanting. Deepwater rice production is practised on 6.5% of the total rice lands in south and southeast Asia.

The most difficult method of cultivation occurs in the upland producing areas. The crop is grown in well-drained soils on level and sloping lands that are prepared and direct seeded under dry, aerated conditions (De Datta 1981, IRRI 1994). Major problems for upland rice production are rainfall and availability of nutrients. Upland rice is more prone to weed competition and total crop failures may occur if weeds are not controlled. Upland rice is grown on 8.3% and 69.7% of the total major rice producing area in Asia and Latin America, respectively (IRRI 1991c). It is also dominant in Africa.

1.5 Rice Blast

There are many diseases of rice caused by fungi, bacteria and viruses. Several important diseases caused by fungi are brown spot (*Cochliobolus miyabeanus* (Ito and Kuribayashi) D.), sheath blight (*Rhizoctonia solani* Kuhn), stem rot (*Magnaporthe salvinii* (Catt.) Krause and Webster) and rice blast (*Magnaporthe grisea* (Herbert) Barr). One of the most important diseases is rice blast.

1.5.1 Importance and Distribution

Rice blast is found wherever the crop is grown, with severity depending on many factors such as cultural techniques, cultivars and climate (Ou 1986). The importance of the disease increased with the development of semi-dwarf varieties and their dependence on nitrogen fertilizers (Atkins *et al.* 1965).

Rice yields may be severely affected by rice blast. Each 1% increase in leaf blast severity (or diseased leaf area) before panicle exertion led to an increased yield loss of 1% (Torres and Teng 1988, 1991). Padmanabhan (1965a) estimated that for each 1% increase in neck blast incidence (or proportion of panicles with infected neck nodes), there was a 0.98% and 0.40% yield loss in susceptible and resistant cultivars, respectively. In Japan, regression analysis gave estimates of 0.57% yield loss per 1% increase in neck blast incidence (Katsube and Koshimuzu 1970) and 0.69% yield loss per 1% increase in panicle blast incidence (Goto 1965). In field experiments on upland rice, yield losses of 4 to 74% were estimated on plots with a range of low to severe panicle blast (Marmolejo 1983). In greenhouse and field studies, yield loss increased with lesion length on the neck which was associated with the time of infection after flowering (Prabhu 1982, Tomio 1988, Vingnanakulasingam 1991, Roumen 1993).

1.5.2 Symptoms

Lesions are produced on all parts of the plant; on the leaves, nodes and internodes, panicles, and occasionally on the leaf sheath (Fig. 1.1). There are several types of lesions based on the susceptibility of the plant (Ou 1985). Susceptible-type (or compatible) lesions are elliptical with pointed ends, grey centered with purple or brown margins, and 1-1.5 cm long and 0.3-0.5 cm wide. Extremely susceptible plants have grey lesions with no clear margins, sometimes with a chlorotic zone around the lesion. Lesions may coalesce, which may lead to leaf death. Severe infections result in stunting of the plant. Lesions on the neck node (the node at the base of the panicle) or neck (Fig. 1.1B) may girdle the stem which results in death of the panicle. Panicles are often broken at the neck node. Resistant-type (or incompatible) lesions are typically brown in color, pinhead to a few mm^2 in size. On a particular leaf of susceptible cultivars, both susceptible and resistant lesions may be observed.

1.5.3 The pathogen

The pathogen, often referred to as *Pyricularia oryzae* Cav., is correctly named *Pyricularia grisea* Sacc. (Rossman *et al.* 1990). Although the former name has been popular, the latter should be used by convention because it is the earliest name given to the pathogen and is commonly used for the pathogen on other grasses (Rossman *et al.* 1990). Furthermore, specimens from different hosts are morphologically indistinguishable and some grass species are susceptible to isolates from rice. The teleomorph, *Magnaporthe grisea* (Herbert) Barr, is rarely found in nature (0u 1985).

The fungus is characterized by simple or sparely-branched conidophores with 2-4 septa. The conidiophores emerge solitarily or in clusters through the stomates or cuticle. They are slightly swollen at the base, tapering towards the apex. One to twenty conidia are borne on short truncate denticles at the apex. The condia are pyriform to obclavate, 2-septate, and sometimes slightly constricted at the septa. Conidial sizes varies with culture conditions and host (Ou 1985), and range from 14 - 32 µm in length by 6.0 - 15 µm in width.

Perithecia of the perfect stage are nonstromatic, with a spherical to subspherical base which is partially embedded in the host tissue and has a long neck. The asci are eight-spored, cylindrical to clavate, and mostly 60-90 µm in length by 5-7 µm in width. Ascospores are fusiform, curved and rounded at the ends, 3-septate, and 18-23 µm in length by 5-7 µm in width. At maturity, the ascospores are extruded from the ostiole in a gelatinous mass.

1.5.4 Life cycle

Mature conidia become airborne and lodge on the aerial parts of rice plants by sedimentation and impaction (Pinnschmidt 1989). Under suitable conditions, the conidia germinate. Germ tubes are mostly formed from the end cells of the conidia, rarely from the middle cells (Ou 1985). At the end of a germ tube, an club-shaped appressorium is formed and adheres to the surface of the plant part. An infection peg is formed and penetrates the epidermis by hydrostatic pressure in the appressorium and enzymatic action. Upon successful penetration, the fungus begins to colonize the host tissue. After a latent period depending on temperature, conidiophores are produced upon which the conidia are borne. The complete cycle requires a minimum of 3-4 days.

Although the sexual stage of the fungus is rare, some isolates from rice produced mature perithecia after mating with hermaphrodite strains from *Eleusine coracana* Gaertn. and *Phalaris arundinacaea* L. (Yaegashi and Nishihara 1976). Subsequently, mature perithecia were formed with matings of rice isolates from distant countries (Kato 1977, Leung *et al.* 1988).

1.6 Epidemiology of Rice Blast

1.6.1 Source of inoculum

Pyricularia grisea has been isolated from spikelets and embryos of infected seeds of rice (Zad and Zakeri 1981). However, the frequency was rare. (Bernaux 1981). In one study, approximately 0.8% of 261 seedlots contained an average of 1% infected seed (IRRI 1991b, 1992). Although plants that arise from infected seeds usually die, they could serve as inocula to initiate primary foci. In Japan, infected seeds have been used as the inoculum source in greenhouse experiments (Honda and Nemoto 1985).

Although the pathogen may overwinter as mycelium and conidia in crop debris in temperate regions (Ou 1985, Padmanabhan 1965b), overseasoning is unlikely to occur in the humid tropics (Ito and Kuribayashi 1932, Bonman pers. comm.). The ability to overwinter/overseason depends on temperature and relative humidity. However, overseasoning is not important in the tropics because airborne conidia are found year round (Ou 1985)

The importance of conidia arising from weeds within the crop, or from other hosts, near the crop is unclear. Itoi *et al.* (1979) determined that the fungus could overwinter on bamboo and isolates from bamboo were able to infect rice seedlings. Isolates obtained from rice were able to infect other crops such as maize, wheat and barley (Andersen *et al.* 1947). Inoculation experiments using isolates from leaves of non-rice plants were both positive and negative (Itoi *et al.* 1979, Kato and Yamaguchi 1980, Mackill and Bonman 1986, Kingsolver *et al.* 1984). Isolates from rice were not always able to infect other grass and sedge species (Paje *et al.* 1964, Itoi *et al.* 1979, Mackill and Bonman 1986). It is possible that infection did not occur for many cross-inoculations because isolates did not possess virulence genes for compatibility.

Using genetic analysis, Borromeo *et al.* (1993) demonstrated that restriction fragment profiles of mitochondrial DNA and nuclear DNA obtained from isolates on rice were different from those obtained from non-rice plants. Profiles of isolates obtained from several rice cultivars were similar. It was observed that whereas some weeds in a rice field were infected, rice plants in the vicinity of the weeds were disease free. They concluded that inoculum from weeds and other species was not important in initiating an epidemic, but acknowledged that more profiles from isolates from these and other species must be observed.

In temperate regions, the source of primary inoculum was infected straw and seed (Akai 1974). In the tropics and subtropics, an important source of inoculum could be from distant sources or nearby rice plantations which are planted sequentially in time. Conidia have been collected in the stratosphere (Suzuki 1975).

1.6.2 Infection

On the leaf blade, bulliform cells and stomata were the main sites for infection (Hashioka *et al.* 1967, Kim 1987, 1986). Appressoria were formed most frequently on bulliform cells [30-44%] and stomata [15-27%] (Kim 1986). Penetration occurred most frequently through these points of entry and accounted for an average of 83% of all penetrations If bulliform cells are cells are silicated, no infection occurred. Stomata were infected with a frequency of 15 to 27%. Other cells were infected with lower frequencies (Kim 1987).

Leaf wetness is required for all of the infection subprocesses; usually the relationship between infection and leaf wetness is well known (Ou 1985, Kato 1974a, Liang 1979). Regardless of other environmental factors, germination begins within 3 hours of deposition, if leaves are wet (Kato 1974a). If conidia pass through a dry period of 24 hours, germination is delayed. After 1 hour, germination was only 8% for dried spores, but 92% for spores kept in a moist chamber (Kingsolver *et al.* 1984). Once spores have been wetted, germination is reduced by drying and few conidia germinate after a dry period of 24 hours (Suzuki 1975). Lesion number, after inoculation with dry spores, decreases on plants receiving up to 2 hours sunshine than on shaded plants (Hashimoto 1981). However, exposure of inoculated plants to sunshine or shade caused a 90% reduction in lesion number relative to controls which were placed in dew chambers immediately after inoculation (Hashimoto 1981).

The effect of interrupted wet periods varies according to when the interruption occurred (Hashimoto 1981, Rahnema 1979). In one study, inoculated plants were subjected to different periods of drying following misting (Hashimoto 1981). After the drying period, the plants were again subjected to misting. Lesions were observed on plants subjected to a minimum of 9 hours of misting. If inoculated plants were removed from the mist chamber after 6 hours and then kept dry for periods of 30 minutes up to 6 hours, no lesions were recorded after plants were returned to the mist chamber. No reduction in infection occurred when inoculated plants exposed to misting for up to 1 hour were subjected to drying for up to 6 hours to remove the free water on the leaves. However, after 3 hours of misting of inoculated plants, the lesion number decreased with increasing exposure in the dry environment; plants exposed to dry conditions for 6 hours had no lesions. After 9 hours or more of misting, there was no decrease in lesion number for any period of drying although increasing the misting period from 9 to 15 hours increased the number of lesions. Increasing the leaf wetness period from 12 to 15 hours increased the infection frequency by 30% (Kato 1976).

In the tropics, the relation of number of lesions/seedling (Y) to dew period (t) was

$Y = -4.0 + 1.92\ t$ ($r = 0.88$, El Refaei 1977).

In Japan, the proportion of conidia which formed appressoria was related to the number of hours leaf wetness after midnight (t) by the linear equation

$A = 3.4\ t - 19.8 \quad [6 \leq t \leq 12]$ (Suzuki 1969).

The number of lesions/100 cm^2 increased with leaf wetness period up to an asymptotic value which depended on cultivar and isolate (Yeh and Bonman 1986). For a given cultivar, the time required to reach a level of 50% of the asymtotic relative infection efficiency varied with isolate.

In upland rice, longer dew periods have been postulated to play an important role in the higher incidence of blast compared with lowland conditions (Ou 1985, El Refaei 1977). Plant density and fertilizer applications may also increase the dew periods (El Refaei 1977, Chien 1984, Suzuki 1975, Kurschner *et al.* 1992). Average dew periods were 12.8, 11.0 and 8.9 hours in stands with spacings of 10 cm X 10 cm, 20 cm X 20 cm and 40 cm X 40 cm, respectively (El Refaei 1977). Plant densities of 120, 60, and 30 hills/3.3 m^2 led to relative dew periods of 1.0, 0.91 and 0.70, respectively (Suzuki

1975). The amount of dew formed was reported to have variable effects on the rate of infection (Kingsolver *et al.* 1984).

The length of the leaf wetness period required for infection is influenced by temperature (Kingsolver *et al.* 1984, Kato 1974a, Ou 1985). The minimum period of leaf wetness required to initiate appressorium formation was 4-6 hours (Suzuki 1975). Appressoria formation required an average of 11 hours at 24 °C (Kato 1974a). The optimum temperatures for appressoria formation have been reported to be 15-18 °C (Ito and Kuribayashi 1931), 15-20 °C (Rahnema 1978), 16-25 °C (Suzuki 1975), and 25 °C(Kato 1974a). The average time for the formation of mature appressoria (A_m) as a function of temperature (T °C) was

$$A_m = 49.7 - 3.34\ T + 0.072\ T^2$$ (Yoshino 1971a).

Appresoria formation was rare at temperatures exceeding 28 °C. The minimum dew period (DP, hours) required for infection as a function of temperature (T) was defined by Barksdale and Jones (1965) as

$$1/DP = 0.265 - 1.452/T$$

This equation was the basis of a forecasting model. The relation of infection (Y) to temperature (T °C) was determined by Yoshino (1971) as

$$Y = 0.062\ T^2 - 2.98\ T + 48.21$$

Using several data sets compiled from the literature, Kato (1974) determined that a minimum dew period of 6-8 hours will initiate infections at the optimum temperature of 25 °C. In contrast, Kapoor and Singh (1977) reported that 12 hours of leaf wetness was required for infection at 20 °C and 25 °C, and 16 hours at 18 °C. Although optimum temperatures were similar, other factors such as cuticular resistance, leaf age and plant age could affect the minimum leaf wetness period. Nevertheless, in the tropics, night temperature is not a significant factor as night temperature is almost always in the optimum range (El Refaei 1977, Ou 1985).

Adhesion of conidia to the plant surface is an important factor in determining appressorium formation (Suzuki 1975). This could be important in periods of extended wetness due to rainfall. In a laboratory study, a teflon surface was sprayed wth a conidial suspension in distilled water. This surface was subjected to washing with distilled water at different times after application of the conidia. The number of conidia removed from the teflon surface decreased from 2000 at the time of application to 300 over a period of 15 minutes (Hamer *et al.* 1988). Although no studies have been conducted using leaves, these results suggest adhesion is unlikely to occur for conidia released and deposited onto a leaf during a heavy rainfall.

Suzuki (1975) found a clear positive correlation between disease severity and number of rainy days in the growing season. This was attributed to extended dew periods and adhesion of spores trapped in rain drops. In contrast, Asai *et al.* (1967) reported that lesion number was negatively correlated with amount of rainfall shortly after inoculation possibly because of spore removal. In the tropics, rain is not an important factor as rainfall usually occurs as heavy showers for a few hours whereas in temperate regions, long periods of drizzle is favorable for infection (Suzuki 1975). This difference between regions could be due to conidia removal from plant surfaces during heavy rainfall (Suzuki 1975).

Under ideal conditions, 0.68% of appressoria penetrated the epidermis of which 51% formed hyphae in the epidermal cells and 17% of the latter were able to infect surrounding cells (Sakamoto 1968, cited by Suzuki 1975). After 24 hours, Liang (1979) reported that 4, 25, 26, 37, 24, 10 and 0% of conidia had produced appressoria and penetrated at constant incubation temperatures of 12, 16, 20, 24, 28, 32 and 36 °C, respectively. In a separate study, the number of penetrations increased with incubation time over a range of temperatures (Yoshino 1979). The average time for penetration was 14.7, 12.2 and 14.0 hours at 18, 24, and 29 °C, respectively. At 24 °C, 0.6% and 2.1% of appressoria had successfully penetrated the epidermis after 6 and 9 h, respectively. In another study, Rahnema (1978) estimated an infection efficiency of 1.6%.

The effect of temperature on infection is modified by its effect on the host. In growth cabinet experiments, the average percent penetration for 6 isolates and 6 cultivars in all possible combinations were 1.37, 0.73 and 0.39 for pre-inoculation diurnal temperature regimes of 29/21 °C, 32/24 °C and 35/27 °C, respectively (Kim and Crill 1980). After inoculation, the plants were placed in a growth cabinet at 25 °C. Mean relative infection efficiencies, the number of lesions per unit leaf area, increased with pre-inoculation temperatures of 20/16, 24/20, 28/24 and 32/28 °C (Yeh *et al.* 1989). Most responses were approximately linear over the average of the day and night temperatures, with rate of increase due to temperature depending on cultivar and isolate. In contrast, when plants were subjected to temperature regimes for 3 days both before and after inoculation, penetration was 2-10 times greater on leaves of plants incubated at 29/21 ^{o}C than at 23/15 ^{o}C (Kim and Mogi 1985). There appeared to be a high isolate X cultivar X temperature interaction.

Plant susceptibility is affected by pre-inoculation enviromental conditons. Exposing plants to low temperature prior to inoculation increased the number of appressoria and the number of cells penetrated compared to control plants exposed to normal or higher temperatures (Suzuki 1975, Sadasivan *et al.* 1965). In contrast, lesion number was reduced in inoculations immediately after pre-inoculation exposure to low temperatures (Yeh *et al.* 1989, Goto *et al.* 1966). Exposing normally resistant cultivars to low night temperatures before inoculation resulted in susceptible reactions on some cultivars (Manibhushanrao and Day 1972). The response varied with cultivar, duration of low night temperature and number of days plants were treated. However, it is possible that the differences were due, in part, to differences in plant and leaf development as treated plants were 10-15% shorter than controls grown at 30 ^{o}C. In another study, the effects of low air temperature depended on time after treatment (Ohato *et al.* 1966); plants were relatively resistant immediately after treatment but increased in susceptibility 6 to 18 day after the temperature treatment. This reversal in susceptibility corresponded to changes in nitrogen content of the leaves.

Plants subjected to low soil temperatures are more susceptible to disease(Otani 1959, Ohata *et al.* 1966, Kato 1983). Furthermore, leaves formed before the low temperature treatment (17-18 ^{o}C) were more susceptible than the control (23-24 ^{o}C), and leaves formed after the low temperature treatment were less susceptible (Katsube and Tokunaga 1963). This response was negatively correlated with leaf water content and positively correlated with leaf nitrogen content.

Sunlight decreased spore germination and appressorium formation of dry and wet spores (Kapoor and Singh 1977). Spores collected from lesions at 07:00 had higher germination rates than spores collected at 12:00. However, no differences were recorded between clear and cloudy days. The rate of germination was one-half the rate in the dark. It was also determined that light inhibits germ tube elongation. Sunlight apparently inhibits appressorium formation (Hashioka 1965). In contrast, Yoshino and Yamaguchi (1974) reported that germination and especially appressorium development was reduced under simulated shade conditions compared to the no shading control. Penetration was greater on plants after removal of shade than on plants kept under shaded conditions. This was attributed to a greater rate of disappearance of dew under shaded conditions but is probably due to lower dew deposition rates under shaded conditions. Pre-inoculation periods of cloudy or partially-cloudy conditions appeared to increase background infection rates but cloudiness prior to inoculation with a spore suspension had no effect on lesion numbers (Asai *et al.* 1967).

Penetration rates are conditioned by plant age, nitrogen and silica applications (Kim and Lee 1982, Yoshino 1979, Volk *et al.* 1958, Kahn and Libby 1958). Penetration rates increased with nitrogen application and decreased with silica application and plant age. Penetration rates of 21, 14 and 7% were estimated at the tillering, panicle initiation and boot stage, respectively (Yoshino and Yamguchi 1974). Lesion number per leaf decreased with plant age and the rate of decrease with plant age varied with cultivar (Kozaka 1979, Torres 1986, IRRI 1985a).

Nutrients also had an important role in the infection process. Spore germination and appressorium formation were stimulated in plants fertilized with high levels of nitrogen or potassium (Kawamura and Ono 1948). Dew obtained from these plants differed in nutrient content. In another study, the quality and quantity of leaf exudates differed between cultivars (Hashioka and Ikegami 1963, Mohanty and Gangpadhyay 1981). Germination was greater in drops of water used to rinse leaves than in distilled water. Rinses from leaves of plants at lower temperatures supported greater germination rates than rinses from plants at higher temperatures. However, pre-infection processes were not always enhanced by nitrogen applications (Kaur *et al.* 1979).

Application of nitrogen resulted in increased infection (El Refaei 1977, Ou 1985, Roumen 1993, Otani 1958, Hashioka and Ikegami 1963, Zsoldos and Vamos 1982, IRRI 1982). Most available data are, however, largely qualitative. In hydroponic culture, susceptibility to blast was greater with nitrate fertilizers than with ammonium fertilizers (Osuna-Canizalez *et al.* 1992). Plants fertilized with higher levels of nitrogen had fewer silicated epidermal cells (Ou 1985, Kim *et al.* 1977). The response of infection to nitrogen applications was linear (Ganguly *et al.* 1954, Farias *et al.* 1982); however, because the response was measured as lesions per leaf as opposed to lesions per leaf area, these results may be biased since leaf area increases with nitrogen application. The effect of nitrogen application on infection varied with cultivars (Padmanabhan 1965b). Disease severity was decreased by split applications of nitrogen (Amin and Venkatarao 1979, Kuerchner *et al.* 1992). Increasing nitrogen does not always increase infection, however. In Florida, soil nitrogen was of minor importance when meteorological conditions were favorable for infection (Beier *et al.* 1959). It is possible that greater blast severity in Korea relative to the Philippines resulted from greater nitrogen application in the former country, although several other factors (cultivars - japonica vs indica, virulence patterns, environmental conditions - tropical vs temperate) were also important (Bonman *et al.* 1989)

The amount of silica in the plant tissue has been correlated with reduced disease severity (Kurschner *et al.* 1992). Infection rates were lower in plants with high levels of silica, possibly as a result of a mechanical barrier resulting from the accumulation of silica in the epidermis (Volk *et al.* 1958). Histological studies showed that silica layers was embedded within the cellulose matrix (Yoshida *et al.* 1962). Silica uptake in several plant parts increased and infection efficiency was decreased over time (Yoshida *et al.* 1962b, Volk *et al.* 1958). Silica uptake was negatively correlated with nitrogen uptake (Osuna-Canizilez 1992, Kurchner *et al.* 1993, Volk *et al.* 1958). The number of silicated cells decreased with nitrogen application (Kim *et al.* 1977). Silica uptake was reduced and nitrogen content greater under magnesium deficiency (Ishizuka and Hayakawa 1951). The resistance of four cultivars to leaf or neck blast was correlated with tissue silica content (Rabindra *et al.* 1961). Drought stress may affect silica content in plant tissue (Ou 1985).

According to Kim (1987), pre-infection processes are not affected by plant water stress. However, conidia arising on drought-stressed plants may behave differently than conidia arising from lesions on non-stressed plants, as has been demonstrated for powdery mildew (Wyness and Ayres 1985).

Plants infected with ufra nematodes (*Ditylenchus angustus* (Butler) Filipjev) prior to inoculation had greater lesion numbers than uninfected plants (Mondal *et al.* 1986). Tissue analysis indicated differences in nitrogen, phosphorus, sulfur and zinc contents between nematode-infected plants and the controls.

Differences in infection rates varied with different soil types (IRRI 1991a, Singh *et al.* 1994a 1994b, Breikaupt 1989, Chuke *et al.* 1979). In one study, this was attributed to nutrient deficiencies in different soils (Chuke *et al.* 1979).

1.6.3 Relative infection efficiency

Relative infection efficiency, defined as the number of lesions formed per unit leaf area, integrates the pre-infection processes and post-infection colonization. The relative infection efficiency is a crude measure since the number of conidia inoculated onto a leaf surface is not known. This measure has been reported more often because observations on penetration and initial colonization are difficult because microscopic observation is hindered by the presence of silica in the epidermal layer. . As a result, it is not known with certainty what stage of the infection process is most affected by the treatments. Liang (1979) reported that appressoria which had penetrated the leaf epidermis were lighter in color than appressoria which had not penetrated the epidermis. However, this observation has not been confirmed by other investigators. In many studies, the inner leaf sheath has been used in many studies to examine penetration. In studies assessing individual processes using leaf sheaths, the greatest differences were observed during the colonization stage (Kaur *et al.* 1974, 1979). Similar results were observed on leaves in a few studies (Peng *et al.* 1986, Peng and Shishiyama 1988, 1989). Results from inoculations on leaf sheaths have been correlated with disease on leaves and in the field (eg. Kim and Crill 1980, Izadyar 1980), but these results are not useful for modelling.

1.6.4 Colonization

After the infection hypha has penetrated the epidermis, subsequent establishment and colonization depend on a number of factors, including genetics, host age, crop nutrition, temperature and soil moisture.

Colonization can be affected by length of the post-inoculation daily leaf wetness duration. Incubation period decreased and lesion size increased with increasing duration of leaf wetness (El Refaei 1977). In another experiment, however, incubation period remained constant over different dew periods except for a dew period of 24 hours which increased the incubation by 1 day at all temperatures. Lesion size increased linearly with daily dew periods of 4 to 20 hours (El Refaei 1977) and slopes increased with temperature. However, lesion number decreased when inoculated leaves were subjected to a daily dew period of 24 hours with numbers equal to 4 hours of daily leaf wetness durations (El Refaei 1977). Lesion number increased linearly with increasing relative humidity from 50% to 95%, but lesion sizes were slightly smaller at high relative humidities.

Incubation periods, lesion size, and lesion number vary with temperature. Hashioka (1950) reported incubation periods of 6, 4, 5 and 6 days at 19, 26, 28 and 32 ^{o}C, respectively. Kato and Kozaka (1974) summarized their data by graphs depicting lesion development over 25 days as a function of temperature, and showed that lesion size was affected by temperature. Lesion growth rate and size was greatest at 25 ^{o}C whereas lesion growth was least at 15 ^{o}C. Similar results were obtained by El Refaei (1977). High alternating temperatures of 32/25 ^{o}C and 32/20 ^{o}C and high constant temperatures of 30, 32, and 35 ^{o}C resulted in high initial lesion growth, with a leveling off at 15 days (El Refaei 1977). Lesions under alternating temperatures responded differently than lesions grown under constant exposure to the average temperature (Kato and Kozaka 1974, El Refaei 1977). The response to temperature was not linear but parabolic. It is possible differences in experimental conditions affects host resistance. Although growth of the pathogen is enhanced by increasing temperatures, high temperatures also increased plant resistance (Suzuki 1975). In contrast, at low temperatures, where the growth of the pathogen is slow, the plant is relatively more susceptible. Lesion number per leaf decreased with increasing temperature after inoculation and the response varied with cultivars (Kozaka 1979). One method used to measure colonization is an index based on the number of cells colonized by the fungus (Yoshino 1972). The degree of tissue colonization was 31.4 at a diurnal temperature of 23/15 °C for a period of 3 days prior to and after inoculation;, 131.4 at 29/21 °C; and 111.4 in a greenhouse with a temperature range of 23-33 °C (Kim and Mogi 1985).

Differences among cultivars were found in the penetration and establishment phase whereas no differences were found in pre-infection processes (Kaur *et al.* 1974, Koga *et al.* 1986). On inner leaf sheaths, establishment was the most important stage when incompatible and compatible isolates were tested (Kaur *et al.* 1973). No differences in the pre-infection processes were observed and only small, insignificant differences were found during penetration. On leaf blades of young seedlings, results are contradictory for different race-cultivar combinations. Fewer penetrations were observed with incompatible race-cultivar combinations than compatible race-cultivar combinations (Tomita and Yamanaka 1983). In another study, there were no differences in germination (96-97%) or appressoria formation (93-95%) but penetration was similar for three of four cultivars (Peng and Shishiyama 1988). Penetration rates were 6-14% after 24 hours, 16-30% after 48 hours and 28-32% after 96 hours; no clear association between penetration and cultivar resistance was evident. This was confirmed in another study involving 13 cultivars (Peng and Shishiyama 1989). Penetration varied with isolate, leaf age and cultivar. Cultivar differences were found for lesion size (Roumen 1993, Roumen 1992, Yeh and Bonman 1985). Sugar content and phenolic production after infection varied with resistance of the cultivars (Jayachandran-Nair and Chakrabarti 1980).

Lesion development can also be affected by plant nutrition. Although small increases in penetration of leaf sheaths occurred with nitrogen applications, larger differences were recorded in the establishment phase (Kaur *et al.* 1979). Applying nitrogen to potted plants one day before inoculation increased the mean relative infection efficiency by 150% thus indicating that nitrogen probably affected post-inoculation processes (Roumen 1993). The effect of applying nitrogen one day before inoculation on lesion size varied with cultivar (de Boef 1989). The effect was more pronounced for maximum lesion size than average lesion size. Nitrogen fertilizer increased lesion size partly because of reduced enzyme activity resulting in lower phenolic and lignin contents (Matsuyama 1975, Matsuyama and Dimond 1973, Otano 1960). The amount of hemi-cellulose in the leaf was reduced and sugar content was increased. A close correlation was often observed between disease severity and soluble nitrogen content under various environmental and cultural conditions (Kozaka 1965). For example, low temperature decreased the C:N ratio and increased free amino acid content (Hashioka and Ikegami 1963). Nitrogen content and other nutrients also varied with seeding density (Amin and Katyal 1979).

Although nitrogen content has often been correlated with lesion development, other essential elements also may be important. The effect of phosphate on disease development depends on soil fertility. For example, phosphate fertilizers reduced disease severity on phosphorus deficient soils, but the addition of phosphate increased disease severity on high nitrogen soils (Ou 1985, Okamoto 1950). The effect of low soil temperatures could be related to the reduction in phosphate uptake (Okamoto 1950). Application of potassium reduced disease severity in some reports and increased disease severity in other reports (Ou 1985), and the effect sometimes depended on the interaction with other nutrients such as nitrogen and magnesium. The relationship of blast severity to plant nutrition is more complex because of the interactions between nutrients in the tissue.

Drought stress increased lesion number, lesion size and disease severity (Bonman *et al.* 1988, Gill and Bonman 1988, Lee 1981, El Refaei 1977, Kahn and Libby 1958). Plants that were subjected to severe water deficit and showed severe leaf rolling had relative infection efficiencies more than 4 times those of unstressed controls (Gill and Bonman 1988). Twenty-two days after inoculation, lesions on stressed plants were at least twice as large as those on unstressed plants. Sugar content and phenolic content were less in infected plants under upland conditions compared to lowland conditions (Kim 1985). Because irrigation and nitrogen interact to decrease leaf water potential at low irrigation levels (Aragon and De Datta 1982), these factors may interact further to increase plant susceptibility.

Fewer acute susceptible type lesions were found on plants under simulated shade imposed after inoculation than in open conditions (Yoshino and Yamaguchi 1974). Also, leaf blast was more severe under pre-inoculation shading than under post-inoculation shading. Acute lesions were more numerous on plants inoculated 5 days after a 10 day shading treatment than on plants with no pre-inoculation shading. A moderate number of acute lesions were recorded on plants inoculated immediately after removal of the lawn cloth after 10 days of shading. Mycelial growth in detached sheaths and lesion size on leaves depended on duration of pre-inoculation shading. Lesions on plants with post-inoculation shading were smaller than on plants with pre-inoculation shading (Yoshino and Yamaguchi 1974, Imura 1938). Shading increased total nitrogen, water soluble nitrogenous compounds, and chlorophyll, but decreased sugar, starch and phenolic content.

1.6.5 Latency

Latent period is the time required from infection to the commencement of sporulation (Zadoks and Schein 1979). It is commonly measured as the time required after inoculation for 50% of the lesions to sporulate. Latent period (P days) in rice blast was related to temperature (T °C), but also depended on the growth stage of the host (Yoshino 1971a)

$P = 16.3 - 0.056\ T$ (sixth leaf stage)
$P = 20.8 - 0.600\ T$ (panicle emergence)

Another equation for latent period in the seedling stage was developed by Sekiguchi and Furuta (1970)

$$P = 113.6/(T-2.5)$$

Latent periods varied from 13 to 18 days at 9-11 °C, to 4-6 days at 26-28 °C (Sekiguchi and Furuta 1970).

In panicle blast, latency is determined by the type of tissue attacked. When temperatures ranged from 16 to 34 °C under natural conditions, the latent period was 6-9 days on spikelets, 7-12 days on panicle axes, and 10-14 days on neck nodes (Kato and Sasaki 1972). At 26 °C, latent periods were 7.5-11.3 days on spikelets and panicle branches, and 8.3-13.1 days on neck nodes (Kato and Kozaka 1974).

In a small sample of varieties developed at IRRI, the observed differences in latency were not considered to be important in determining disease progress although there were differences in the infection efficiency (Roumen 1993). Similar conclusions were obtained in a study comparing Korean cultivars (Yeh and Bonman 1986).

However, in a study comparing 69 cultivars, mean latent periods of 5.7, 6.7 and 8.5 days were determined for susceptible, moderately susceptible and moderately resistant groups (Castano *et al.* 1989). There was considerable overlap in the distribution of latency among groups; the smallest overlap occurred among the extremes in resistance groupings. However, there was no indication of the lesion type, an important consideration when searching for partial resistance (Parlevliet 1989). No significant differences were evident in latency as a result of pre-inoculation drought stress (Gill and Bonman 1989).

1.6.6 Sporulation

Mature lesions are capable of sporulating when relative humidity (RH) is higher than 89%, with an optimum RH > 93% (Hemmi and Imura 1939). Under optimum conditions, conidiophores were formed in 4-6 h, with one conidium formed in 40 min (Toyoda and Suzuki 1952). El Refaei (1977) reported that sporulation occurred at 50-80% RH, although numbers were small. Conidial production over 30 days was greater at 95% RH. Spore production was highest on the sixth day after lesion appearance (Villareal *et al.* 1980), although a separate study showed that the time of maximum production varied in controlled conditions (El Refaei 1977). Kato *et al.* (1970) reported that maximum sporulation occurred 7-12 days after inoculation, although sporulation continued for 60 days (Suzuki 1975). In experiments conducted by El Refaei (1977), no spores were formed after 30 days.

Sporulation is dependent on air temperature (Kato and Kozaka 1974, El Refaei 1977). Maximum sporulation occurred on the ninth day after inoculation at 20 °C, between the third and ninth day at 25 °C, and on the third day at 32 °C. However, sporulation occurred between the third and fifth day at alternating 12 hour temperatures of 32/25 °C and 32/20 °C and on the fifth day at 25/15 °C. Sporulation was highest at a constant temperature of 20 °C (6,500 spores/lesion) and at an alternating temperature of 25/16 °C (3,900 spores/lesion; Kato and Kozaka 1974). The minimum, optimum and maximum temperatures for sporulation were 9-12 °C, 25-28 °C, and 34-35 °C, respectively (Henry and Andersen 1948, Kato 1974).

The age and type of tissue colonized affected the sporulation potential (Park *et al.* 1983, Kato *et al.* 1970, Kato 1970). Sporulation is greater in lesions on leaves that were expanding when inoculated than on leaves that were fully expanded 3-4 days before inoculation (Kato *et al.* 1970). Also, number of conidia per lesion varied with leaf position (Park *et al.* 1983). Lesions produced on plants inoculated at the tillering stage had a greater sporulation potential than on those plants inoculated after the formation of the panicle primordium (Kato *et al.* 1970, Park *et al.* 1983). Numbers of conidia varied from 80,000/spikelet lesion to 280,000/neck node lesion. As lesions enlarged, sporulation was greatest along the margins of the lesion (Barksdale and Asai 1961).

Lesion type and size determined the sporulation potential (Dhua 1989). Spores numbered 10,440 on purple grey lesions, 4,680 on yellow purple grey lesions, and 490 on brown grey lesions, each 20 mm^2 in size. Sporulation on purple grey lesions 3, 5, 10 and 20 mm^2 in size was 2770, 4330, 8320 and 10440 spores, respectively. Maximum sporulation was associated with lesions having grey centers and margins changing from dark purple to brown (Kato *et al.* 1970). The time of color change varied with cultivar and age of the leaf part (de Boef 1989).

Sporulation potential is related to the level of partial resistance (Castano *et al.* 1989, Yeh and Bonman 1986). In one study, number of conidia per lesion varied from 4,800 on IRAT13 to 33,000 on IR442-2-58 (Villareal *et al.* 1980).

Preinfection water stress increased the sporulation potential (Gill and Bonman 1988). Lesions on plants stressed to severe leaf roll produced up to 3.5 times more conidia than lesions on nonstressed plants. Furthermore, the infectious period was prolonged by preinfection drought stress.

1.6.7 Spore release and dispersal

In general, high relative humidty or dew is required for spore release under field conditions (Barksdale 1961, Suzuki 1975, El Refaei 1977, Hashimoto 1981, Ou *et al.* 1974, Kim *et al.* 1989). Conidia were released mainly at night with peaks occurring during periods of leaf wetness (Ou *et al.* 1974, Ramalingam 1966, Kim *et al.* 1990) . A second or third peak was observed during monsoon showers in the day (Ou *et al.* 1974, Kim *et al.* 1989). The duration of spore release and peak numbers of spores increased with longer leaf wetness periods (El Refaei 1977). Because the latter observations were taken over a number of days, it is possible that differences were related to lesion development.

Spore release also varied with relative humdity (RH); with more conidia released with increasing RH and no conidia released at RH less than 80% (El Refaei 1977). In addition, initial release was delayed at lower RH, presumably because conidial formation was delayed. Twice as many conidia were released in the presence of free water than at 100% RH (El Refaei 1977, Kim *et al.* 1990).

Spore discharge, from lesions on neck nodes, occurred with rapid changes in relative humidity (Leach 1980). Increasing RH, particularly at or near saturation, was more effective for spore release than decreasing RH and larger changes in RH resulted in greater spore release. The number of conidia released decreased with each cycle of changing RH with the highest release occurring in the first cycle. Infrared radiation had little or no effect. It remains to be determined whether discharge patterns are similar for lesions on leaves.

Simulated shading reduced the number of spores dispersed (Yoshino and Yamaguchi 1974). The authors concluded that this was due to an increase in evaporation of dew under shaded conditions. However, it is more likely that the cotton screen used to simulate the shade had an effect on air turbulence within the canopy and decreased the radiative heat loss from the canopy.

Conidia exposed to wind speeds greater than 1.5 m/sec can be released (Suzuki 1975) perhaps because of leaf movement. Vibrations caused by tapping a spore release chamber resulted in significant numbers of spores being released from lesions on neck nodes (Leach 1980). However, there was an interaction of vibration with relative humidity. Conidia were released more effectively when vibrations were made at RH's less than 50%.

1.6.8 Disease Gradients

The spatial spread of rice blast has been examined by a few investigators (El Refaei 1977, Kim 1987, MacKenzie 1979, Suzuki 1975). Transforming previously-collected disease progress data (from El Refaei 1977), recorded as lesion number/seedling, at different distances from a point source resulted in lines with the same slope but different intercepts. This indicated that over a time-frame of 14 days, disease spreads as an isopath which advances at a constant speed. An isopath is a contour connecting points of equal disease severity. These contours advance outward from the inoculum source as the disease increases (Minogue 1986). This isopath movement for other pathosystems has usually been calculated based on disease severity. Isopath movement, based on logarithms of lesion counts, was greater under upland conditions than in flooded fields (Kim 1987). Isopaths moved at a rate of 0.4 m/day and 0.2 m/day in upland and lowland plots, respectively. This probably reflects differences in plant susceptibility due to cropping management, affecting the amount of inoculum. However, determination of isopath movement in the rice blast pathosystem may lead to biased estimates because susceptibility varies with plant age.

The spread of a disease is dependent on size of initial foci (Zadoks and Schein 1979). This was confirmed for rice blast by Barksdale (1967) where initial foci in the center of three fields were of different sizes. However, the rates of spread were confounded with different numbers of lesions/0.3 m row in these foci at the initial assessment shortly after inoculation.

Although some dispersal may occur by splashing rain, wind is more important. In seasons with little or no wind, the disease was aggregated whereas in seasons with more wind, the disease was uniform (Suzuki 1975).

1.6.9 Disease Progress

The shape of the disease progress curve depends on the disease variable that is being measured as a function of time. Actual diseased leaf area generally progressed unimodally; increasing to a maximum and then decreasing thereafter (Bonman *et al.* 1991, Pinnschmidt 1989) if functional leaf area was considered, and dead leaves were removed. Cumulative lesion number or cumulative conidial counts progressed sigmoidally (Pinnshmidt 1989, Sekiguchi and Furuta 1968).

Disease progress varied among cultivars. In addition to the factors described above, canopy structure may play an important role in disease development (Friedrich *et al.* 1991). In low and sparse canopies, dew formed in the lower part of the canopy and dew formation increased with increasing canopy height and density. The distribution of dew was dependent on the canopy structure. On a cultivar with vertical leaves in a dense canopy, maximum dew was found at the 2/3 height. On the other extreme, on a cultivar with droopy leaves, maximum dew was found on the upper horizontal leaves. The distribution of disease with leaf insertion number paralleled the distribution of dew.

A combination of management techniques may limit disease progress. Using partially-resistant cultivars in flooded seedbeds has been effective in slowing disease progress whereas flooded conditions had no beneficial effects when a susceptible cultivar was used (Sah and Bonman 1992). Using mixtures of resistant and susceptible cultivars has decreased final disease severity (Asaga *et al.* 1983, IRRI 1985b, Bonman *et al.* 1986) and apparent infection rates (Nayak *et al.* 1982). Using mixtures was particularly useful in reducing disease severity under drought conditions in experimental plots (Bonman *et al.* 1986). Area under the disease progress curve was less for epidemics in plots with split application of nitrogen fertilizers than epidemics in plots with a single application (Kuerchner *et al.* 1992) . Applications of herbicides increased neck blast incidence (Singh 1984), possibly as a result of direct effects on rice, more efficient uptake of nitrogen, an increase in tillering, and/or a decrease in number of resistant hosts that serve as spore traps. Planting at different densities affects the leaf wetness period (Chien 1987, El Refaei 1978). Chien (1984) reported leaf wetness periods of 13 and 10 hours at 5 X 5 and 10 X 10 cm^2 spacings, respectively. Little or no dew was recorded at the greater spacings. Unfortunately, no information was provided on leaf area index. Leaf area index is affected by the application of nitrogen fertilizers. Thus, it may be possible to partition the effects of nitrogen into the direct effect on plant susceptibility and the indirect effect on leaf wetness duration.

There are many factors which affect disease progress. Although previous studies have provided valuable information on the mechanics of disease development, many of these data are largely qualitative and, as such, of little value in modelling disease dynamics (Teng *et al.* 1991). Many of these factors have both direct and indirect effects. For example, the interaction of nitrogen and silica has been discussed. In addition, the application of nitrogen increased tiller number and leaf area index (Fagade and De Datta 1971) which could affect leaf wetness periods and water deficits, Leaf angle was found to vary with silica application (Luisa *et al.* 1989) and, thus, silica indirectly affects spore deposition rates and dew formation. Application of phosphorus increased water deficits and disease severity due to increased leaf area index (IRRI 1988).

1.6.10 Resistance

Breeding for resistance to rice blast has emphasized race-specific or complete resistance. That is, one or few genes for complete resistance are incorporated into the cultivar, resulting in hypersensitive, incompatible reactions with avirulent isolates. This type of resistance is not durable since the pathogen population can adapt, and often leads to the typical boom and bust cycle (Bonman and Mackill 1988, Ezuka 1972).

There have been several reports that susceptible-type lesions were produced on some resistant varieties under conditions favorable for pathogen growth. These included high nitrogen (Gang 1986) and low temperature (Manibhushanrao and Day 1972, Lee 1981). However, no studies were conducted to verify the virulence pattern of isolates from lesions produced on these plants.

Partial resistance results in reduced rates of epidemic development, despite the formation of susceptible lesion types (Parlevliet 1979). Partial resistance is a relative measure, a measure that is compared to a standard cultivar (Parlevliet 1979). One or more of the infection components such as infection efficiency, latent period, sporulation capacity, or infectious period differ in cultivars with partial resistance (Parlevliet 1979). The effects of varying one or more of these components on disease progress has been demonstrated through simulation (Zadoks 1971).

Partial resistance is believed to be oligogenic to polygenic, that is, resistance is controlled by a number of minor genes. For example, in the *Hordeum vulgare - Puccinia hordei* pathosystem, a number of minor genes determined a longer latent period which was correlated with partial resistance (Parlevliet 1978).

Partial resistance is thought to be durable. Partial resistance is generally race non-specific. However, some reports indicated that partial resistance may be race-specific (Bonman *et al.* 1989). In a subsequent study, Roumen (1993) showed that only 1% of the total variation was due to the isolate X cultivar interaction. Although the main effects were large, suggesting race non-specific resistance, he also concluded that partial resistance to rice blast was race-specific. That is, minor genes for partial resistance operate in a gene-for-gene relationship with minor genes in the pathogen. Similar systems were reported for barley leaf rust (Parlevliet 1978). Simulations indicated that in pathosystems with minor genes that produced small effects and worked on a gene for gene relationship, behaved as a race non-specific system (Jenns and Leonard 1985).

Resistance based on low relative infection efficiency in the field was determined by the number of major genes in a cultivar (Ahn and Ou 1982a,b). Varieties with a large number of major genes for resistance had the lowest relative infection and thus appeared to have partial resistance. This would be effective where a heterogeneous population of the pathogen occurs. *M. grisea* was reported to be variable and new races were found even in cultures from single spore isolates (Ou and Ayad 1968, Giatgong and Frederiksen 1969). However, others have reported that the pathogen is relatively stable (Bonman *et al.* 1987, Latterell and Rossi 1986).

Rice cultivars with different levels of partial resistance to blast show differences in infection efficiency and lesion development, as measured by lesion size and latent period (Roumen 1993, Yeh and Bonman 1986, Villareal 1981). Average lesion size on Milyang 57 and IR50 was 3 times that on Milyang 42 (Yeh and Bonman 1986). Resistance is modified by leaf and plant age (Andersen *et al.* 1947, Kahn and Libby 1958, Goto *et al.* 1961, Notteghem and Andriatompo 1979, Torres 1986, Kim *et al.* 1987, Roumen 1992). In data from Yeh and Bonman (1986), the number of lesions on the fifth leaf of IR36 was 1/60 the number of lesions on the partially-extended sixth leaf; for IR50, the factor was 1/11. Similar results were observed in a detailed study by Roumen (1993), who concluded that differences in partial resistance in lowland cultivars were due to differences in the rate that resistance increased with leaf age. Although this component of partial resistance may be race specific (Roumen 1993, Kahn and Libby 1958), the cultivar x isolate interaction was small (Roumen 1993). The rate of increasing resistance with plant age also appeared to vary among cultivars (Koh *et al.* 1987, Torres 1986, Kozaka 1979). The age of the panicle at the time of inoculation was an important factor for infection and yield loss (Roumen 1993).

1.7 Rice Blast - Two pathosystems

The disease consists of two phases: leaf blast and panicle blast. Although the pathogen is the same, the dynamics of the two systems are different (Teng *et al.* 1991).

Panicle blast has been correlated with leaf blast severity in some (Bonman *et al.* 1987, Ou and Nuque 1963, Willis *et al.* 1968, Templeton *et al.* 1961, Park *et al.* 1983), but not all studies (Choi 1991, Willis *et al.* 1968). These inconsistencies have been explained by differences in susceptibility to different races (Ou 1985). Different races were found to predominate at different times of the year in a blast nursery at IRRI (Quamaruzzamann and Ou 1970). Other mechanisms are possible (Bonman *et al.* 1989). Different prevailing microclimatic conditions could have occurred at different stages of crop development, favorable for infection at one stage and unfavorable at another. Also, pre-infection microclimatic conditions could alter the susceptibility of the host at different stages of phenological development. Differences in crop development among cultivars could be important by affecting the age and, thus, resistance of the panicles to infection in artificial inoculation trials.

1.8 Research Objectives

The research in this thesis was largely focussed on the effects of drought stress on partial resistance. A major component of partial resistance is the decrease in infection efficiency with leaf development stage and plant age (Roumen 1993, Torres 1986). However, although the infection efficiency increases with drought stress (Gill and Bonman 1988), the effects of drought stress on partial resistance in different plant parts is unknown. Thus, a major objective of this thesis is to determine if the increase in resistance with leaf age and plant age is affected by pre-inoculation drought stress in the vegetative stage of plant development.

An important aspect of the disease cycle is the infection process. The relative number of conidia which developed appressoria, penetrated, and subsequently colonized the tissue determined the relative infection efficiency which increased with leaf wetness duration (Yeh *et al.* 1989). Another objective of my research is to determine the effects of drought stress and leaf position on the rate of infection with increasing leaf wetness duration.

In previous studies, the effects of drought stress on lesion size, latent period and sporulation were determined using low relative infection efficiencies (Gill and Bonman 1988). However, it is possible that these epidemiological parameters may not be relevant for use in rice blast simulators because competition among lesions could alter lesion development and influence latent period and sporulation. Thus, the effect of drought stress on these components of the disease life cycle is re-examined.

Although leaf and plant age effects are important attributes of the epidemiology of the *Oryza sativa-Magnaporthe grisea* pathosystem, the relative importance of these attributes in the epidemiology of the disease is unknown. A general simulation model is constructed to examine the relative importance of these components of partial resistance on disease development.

It is likely that rice blast affects root growth and, thus, affect the ability of the host to withstand a future drought stress event. The effects of rice blast on root growth is examined. The final objective of my research is to determine if there is an interaction between post-inoculation drought stress and rice blast on root growth.

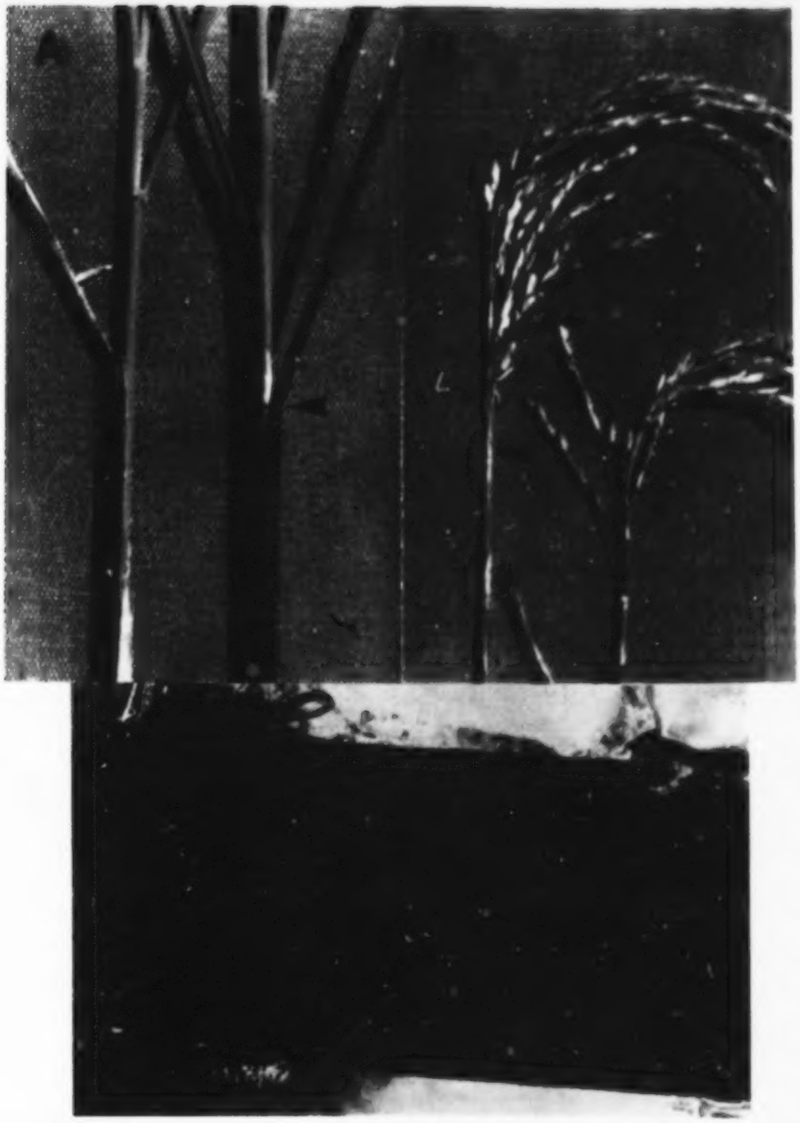

Fig 1.1. Blast symptoms on different parts of the rice plant. A. Leaf collar. B. Neck. C. Leaf (Note the resistant-type lesion on leaf indicated by the arrow).

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Chapter 2. Effect of pre-inoculation drought stress on partial resistance of rice to leaf blast.

2.1 Introduction

Rice is grown in several agroecosystems which include rainfed and irrigated lowland environments and the rainfed upland growing areas. One of the major constraints in the uplands is the availability of water, particularly at panicle initiation. Yields may be severely restricted if drought stress occurs at this stage. In addition, rice blast, caused by *Magnaporthe grisea* (Herbert) Barr, plays a more important role in the rainfed lowlands and the uplands because drought stress may increase the susceptibility of the host (Gill and Bonman 1989, Bonman et al. 1989, El Refaei 1977).

Although susceptible-type lesions are present, disease progress is often reduced in many cultivars. Partial resistance may occur in a cultivar possessing one or a combination of lower infection efficiencies, longer latent periods and low sporulation capacity (Parlevliet 1979, 1989, Zadoks 1971). An important component of partial resistance to the disease in lowland rice cultivars is the reduction in relative infection efficiency with increasing leaf and plant age (Torres 1986, Roumen 1993, Kahn and Libby 1958). The latter was determined to vary in importance with cultivar and to be under oligo- or polygenic control (Roumen 1993). In simulations, this component was effective in reducing the disease epidemic, particularly with rapid increases in resistance with age (Chapter 3).

Partial resistance may be altered by management practises or environmental conditions. In this paper, we examined the role of drought stress in modifying this component of partial resistance to blast.

2.2 Materials and Methods

General methods. All experiments were conducted in a greenhouse at the International Rice Research Institute (IRRI), Los Banos, Philippines, using the following methods, except where noted.

Plants were grown in plastic pots without drainage holes. Silty clay soil (Typic Hapludoll) was obtained from an upland site at IRRI, air dried and sieved to remove large clumps. Equal weights of soil (1.5 kg) were added to all pots in each experiment.

Eight to twelve seeds were planted into each pot and after 7 days, thinned to six to eight plants of uniform physiological age. Physiological age was determined by position and length of the expanding leaf. Plants were fertilized with an ammonium sulphate (50 kg/ha) solution at the time of seeding.

Soil moisture was determined gravimetrically. To determine the pot weight at which severe drought stress occurred, an additional four pots were prepared for each experiment. Water was withheld from these pots until severe leaf rolling (5 on the IRRI leaf roll scale [O'toole and Moya 1978]) occurred. Leaf rolling was reversible when pots were rewatered. Using the pot weights at saturation and severe drought stress as endpoints, the required pot weights for the desired levels of water stress were determined by interpolation.

Soil was maintained near saturation for the first 15 days after seeding for all treatments by adding water, twice daily, to reestablish saturation. At this point, plants were at the third to fourth leaf stage. Thereafter, water was withheld for all treatments except the saturated soil treatment. Intermediate levels of water stress were produced by first allowing the soil to dry until weights reached the level of the desired stress treatment. Subsequently, pots were weighed twice daily and watered to maintain the weight associated with the desired level of water stress. The highest level of water stress was produced by withholding water until leaf rolling occurred. At this time, all pots in the experiment were watered to within 25 g of saturation, and then 25 ml of an ammonium sulphate (25 kg/ha) solution was added. Immediately after this final watering, a fine mark was placed on the expanding leaf at the point of leaf emergence so that it would later be possible to determine what proportion of the youngest leaf had been formed at the time of inoculation. Plants were inoculated in the late afternoon of the same day. Following inoculation, soil saturation was maintained.

Inoculum was prepared following the methods of Mackill and Bonman (1986). Isolates were obtained from the Entomology and Plant Patholgy Division, IRRI. A colonized sorghum seed was placed on a prune agar slant in a test tube and incubated for 7 days. The colony was scraped with a bacterial transfer loop after 10 mL of distilled water had been placed in the test tube. The resulting suspension of conidia and mycelial fragments was poured onto glass petri dishes containing oatmeal agar. The plates were incubated for 7 days at 27 C. The mycelial mat was then scraped with a rubber policeman and the plates, with the lid removed, were placed in a cabinet with a fluorescent light for 3 days. These plates were used for inoculum or stored in a cold room for a period of not more than 3 days. This period of storage was necessary because of uncertainty in predicting when the plants would reach the desired level of water stress. On the day of inoculation, distilled water was added to the petri dish, the mycelial mat scraped with a sterilized rubber policeman or a glass slide, and the suspension filtered through four layers of cheese cloth. The conidia were counted with a Haemocytometer and the concentration of the suspension was adjusted to 1×10^5 spores per ml. An equal volume of a 1% gelatin solution was added to obtain a final concentration of 0.5×10^5 conidia per mL in 0.5% gelatin.

The conidial suspension was sprayed onto the plants, grouped by blocks, until runoff. Plants were placed into a dew chamber at 25 C with one or two blocks per chamber, depending on the number of treatments. Plants were removed from the dew chamber 16 hours later and transferred to an incubation room maintained at high humidity and 25-30 C. Plants were assessed for disease 5-7 days later.

For disease assessment, the youngest three leaves were removed from the plant and mounted using clear tape onto paper. The entire leaf was placed on the tape prior to mounting onto paper. Susceptible- and resistant-type lesions were counted on each leaf and leaf area was estimated.

In all experiments, lesion counts were converted to relative infection efficiencies, (lesions/100 cm^2 leaf). In a preliminary experiment, leaf area was measured with a leaf scanner (LiCOR) and the length and width of individual leaves were recorded. The leaf area was calculated as $0.70 \cdot$ length $\cdot$ width ($R^2 = 0.94$, $P<0.05$). For leaf tips, the area was calculated as $0.5 \cdot$ length $\cdot$ width.

Leaf developmental stage of the expanding leaf of the main tiller was calculated by the proportion of its length at inoculation to its final length. Plant development stage was calculated by adding this proportion to the leaf number of the (n-1)th leaf, where n is the leaf position of the expanding leaf.

Lesion number data were transformed using $\ln(x+1)$, where x is the relative infection efficiency. For the repeated measures analysis, the Greenhouse-Geiser F statistic was used for determining significance in all tests involving leaf position or segment position (Madden 1986). If the analysis of variance revealed significant treatment differences, means were subjected to trends analysis and/or linear or nonlinear regression analysis.

Effect of cultivar and drought stress on relative infection efficiency. Three experiments were conducted in this series. The cultivars C22, Kanong Patong, OS-6 and UPLRi-5 were used. These cultivars showed a range in partial resistance (Roumen, pers. comm.). C22 was very susceptible, Kanong Patong and UPLRi5 were moderately susceptible, and 0S-6 was partially resistant.

In the first two experiments, two pre-germinated seeds were planted into 24 pots (12.5 cm diameter) per cultivar. Seeds were placed in petri dishes with moist filter paper and incubated in the dark at 25 C for 48 hours. Four cultivars and four levels of soil moisture were assigned at random to 16 pots per block. There were 12 blocks in a randomized complete block design.

For the third experiment of this series, eight seeds were sown per pot into dry soil prior to watering to saturation. Seeding was done over a period of 2 days. Pots were thinned to six plants, 7 days after seeding. On the fifteenth day, plants were sorted into four groups according to position and length of the expanding leaf. Each group received a different water stress treatment, with more developed plants being subjected to water stress. This was done so that all plants would be of approximately the same development stage at the time of inoculation. The isolate V850256 was used. Because dew chambers were not available, inoculated plants were placed in makeshift wooden frames, lined with plastic and wet jute bags. Plants were removed after a period of 14 hours, at which time there was no observable film of water on the leaves.

In all three experiments, relative infection efficency of both susceptible- and resistant-type lesions was determined. Susceptible-type lesions have a sporulating grey center with or without a brown to purple border whereas resistant-type lesions are dark brown without a grey center, small in size and nonsporulating (Ou 1985). Both types of lesions may occur on leaves of plants inoculated with a virulent isolate.

Effect of drought stress on relative infection efficiency on leaf segments. In this experiment, only the susceptible cultivar C22 and the isolate P06-6 were used. In addition, clay soil (Orthoxic Palehumult) was obtained from an upland farm near Cavinti, Laguna, Philippines. The soil from this site was highly conducive to the development of blast (Kurschner 1992, Singh et al. 1995). A PVC plastic pipe was placed in pots (12.5 cm diameter) containing a 1 cm layer of soil and the remaining soil was placed around the pipe. Water was added to each pot through the pipe to prevent puddling which would change the soil structure. Twelve seeds per pot were planted to 80 pots and thinned to eight seedlings after 7 days. Pot weights associated with eight different water stress levels were determined. Pots were arranged into 10 blocks in a randomized complete block design.

Fifteen days after seeding, plants (4th leaf stage) were subjected to water stress. Watering was done twice daily, at 8 am and 4 pm. Prior to the morning watering during the stress period, all plants in a pot were marked at the base of the expanding leaf. On the day of inoculation, all plants in a pot were similarly marked in the morning and prior to inoculation. This demarcated the leaf growth that occurred during the stress period into leaf segments and permitted a measurement of leaf elongation.

Beginning two days after susceptible-type lesions were visible, the three youngest leaves were stripped from each plant. Stripping was completed over a period of 3 days and all leaves in the same replicate were removed on the same day. Maximum length and width of each leaf, the distance from the base of the leaf to each felt pen mark and the leaf width at each mark were recorded. Segments at the tip of the nth leaf included the base of the (n-1)th leaf. Susceptible- and resistant-type lesions were counted for each segment.

This experiment was repeated once. In the second experiment, leaf water potential was determined for all treatments, two hours prior to inoculation. The youngest fully-expanded leaf was cut at the base using a razor blade and placed in a pressure chamber (Model 3010I20, Soilmoisture Equipment, Santa Barbara, CA, U.S.A.). The pressure in the container was allowed to increase slowly, using liquid nitrogen as the propellant. The pressure when water began to exude from the cut surface was recorded. One leaf from each of two replicates was measured for each treatment.

Relative infection efficiency was calculated for each segment. Segment area was calculated as the product of the length of the segment and the average width of the segment endpoints. For segments extending to the next lower leaf, the area of the tip of the nth leaf was added to the segment area of the (n-1)th leaf. Segment age was calculated as the number of days from segment emergence to inoculation.

Water stress could potentially have both a direct effect on susceptibility and an indirect effect resulting from changes in physiological age. To test this possibility, the partial correlation, after adjustment for the stress and replicate effects, between leaf development stage at the time of inoculation and the number of infected segments was determined. Similarly, partial correlations between leaf development stage and transformed lesion number were determined seperately for susceptible- and resistant-type lesions on different aged segments.

If there was a partial correlation between the number of infected segments or lesion number and leaf development stage, then the latter term was used as a covariate in a subsequent analysis of the effects of water stress on the former two terms. In the absence of such a partial correlation, leaf development stage was not used as a covariate. Analyses were performed PROC GLM (SAS 1985)

If the effects of stress on lesion number were significant, a nonlinear regression was performed to model lesion number as a function of stress and leaf development stage. The least squared means from the analysis of variance (or covariance) for each segment were retransformed to the original scale and the cumulative infection frequency over segments was determined by rectangular integration beginning with the youngest segment at the time of inoculation. The curves for each stress level were subjected to nonlinear regression. The model consisted of two parts: (a) a cumulative distribution function based on the Richards model (Waggoner 1986) to determine the probability of a lesion occurring on a particular segment and (b) a function determining the total relative infection efficiency over all segments on a plant. The generalized Richards model has been used to model sigmoidal growth functions. The form of the model depends on the value of the shape parameter m (Waggoner 1986). If $m < 1$ then the equation takes the following form.

$$(2.1) \qquad F(t) = \left[\left(1-(1-b)^{1/(1-m)} \right) e^{-rt} \right]^{(1-m)}$$

where b is a constant ($0 < b < 1$), r is the intrinsic rate of the Richards equation and t is the time.

In our model, segment age corresponded to the independent variable t and was defined as (AGE + Q) where Q is a shifting parameter that allowed the function to shift along the AGE-axis without changing the shape parameter. The intrinsic rate of the function was defined as a linear function of stress which was coded from 0 to 7 to represent the eight levels of soil moisture. Thus, the cumulative density function used was

$$(2.2) \qquad RIE_{cum}(Age) = \left[(1-(1-b)^{p})e^{-(k+R \cdot STRESS) \cdot (AGE + Q)} \right]^{1/p}$$

where RIE_{cum} is the cumulative relative infection efficiency, $p = (1-m)$, age is the segment age, k and R are parameters for the Richards rate as a function of drought stress. This function predicts the proportion of total lesions that are on segments 0 to AGE days old.

The second part of the model, the function that determined total relative infection efficiency over all segments, was a piece-wise function. The equation was:

$$(2.3) \qquad A(STRESS) = \begin{cases} c_1 + d_1 \cdot STRESS \text{ if } STRESS < S_O \\ c_2 + d_2 \cdot STRESS \text{ if } STRESS > S_O \end{cases}$$

where c and d are the intercept and slopes of the linear components, S_O was a threshold value beyond which infections were greatly increased and STRESS is defined above.

All parameters were estimated simultaneously with a few exceptions where Q, p or b had to be fixed to avoid nonconvergence and/or singularity. A number of regressions were conducted to determine the appropriate values for p, b and Q on the basis of residual plots and coefficient of determination. The appropriate value for S_O was determined by inspection of the plot for total RIE and stress. Confidence intervals for d1 and d2 were compared to determine if the slopes of RIE vs. STRESS changed at S_O. Overall model adequacy was determined by the coefficient of determination, inspection of residual plots, and the correlation between residuals and the normal distribution. Nonlinear regression analyses were preformed using NONLIN (Sherrod, 1992). First order differences of the cumulative distribution were used to calculate the predicted number of lesions at a given age.

Effect of plant age and drought stress on relative infection efficiency. The same general procedure described as above was followed with two alterations. Larger pots (2.5 kg) were used and groups of four seeds were planted on four different occassions at intervals of 5 days into the same pot, the quadrant selected randomly. Thus, the experimental design was a split-plot with pots as the main plot to which stress treatments were randomly assigned and seeding dates as the subplots. The three youngest leaves harvested from each plant were treated as repeated measures.

Marks were placed at the base of the expanding leaf at the beginning and end of the stress regime. Plants were inoculated with the isolate PO6-6. Six days after inoculation, leaves were removed and placed onto paper as decribed previously.

The data were analyzed by linear regression. The model was:

$$(2.4)\quad \ln(RIE+1)=B_1\cdot(LFNO-1)+[B_0+B_2\cdot STRESS+B_3\cdot LFAGE] + [B_0'+B_2'\cdot(STRESS-6)\ +B_3'\cdot LFAGE']$$

LFNO = the number of leaves on the main tiller

LFAGE = development stage of expanding leaf if stress = S0-S5; and 0 otherwise.

LFAGE'= development stage of expanding leaf if stress = S6-S7; and 0 otherwise.

STRESS = 0-5 for stress levels S0-S5

STRESS'= 6-7 for stress levels S6-S7

B_0 =intercept for stress levels S0-S5

B_0'=intercept for stress levels S6-S7

B_2-B_3, B_2'-B_3'= coefficients for STRESS and LFAGE for stress levels S_0-S_5 and S_6-S_7, respectively.

The division of the stress levels into two groups was determined by significant differences among means from a preliminary analysis of covariance with leaf age as the covariate and seeding-date and stress levels as the independent variables. Analyses were performed using PROC REG (SAS 1985)

2.3 Results

Effects of cultivar and drought stress on relative infection efficiency. Time to leaf roll, on the 9th and 11th day in the first and second experiments, respectively, was greater than for the third experiment in which leaf roll occurred on the 5th day. In the first experiment, the mean length of the youngest leaf was correlated with water stress; saturated plants had longer leaves than stressed plants, but the difference was, at most, 5 cm. In the second experiment, plant growth was highly variable within and between stress levels; for example, nonstressed plants were in the 6th -7th leaf stage, whereas plants were in the 6th leaf stage for stress levels 3 and 4. Youngest leaves within cultivars in the third experiment were remarkably uniform in length at the time of inoculation, but varied slightly among cultivars.

Overall trends were similar for all experiments, but mean relative infection efficiencies differed. In the first and second experiments, there was considerable error variation and no significant water stress or cultivar effects were detected. Several nonleaf-rolled plants had no lesions. Only a few resistant-type lesions were observed on OS-6 in the first two experiments. In the third experiment, no lesions were found on OS-6 which therefore was excluded from the analysis. Only the results of third experiment are reported here.

The main effects of cultivar, stress and leaf position accounted for a large proportion of the model sum of squares (Tables 2.1, 2.2). Lesion number increased with stress on all leaves and increased with leaf position (Table 2.3). Although interactions were significant, only the interaction of cultivar with stress for a large proportion of the observed variation. The effect of drought stress on number of susceptible-type lesions on different leaves also varied with cultivar (Table 2.3). Lesion number on the main tiller of UPLRi5 increased linearly with stress but lesion number increased curvilinearily on C22 and on Kanandong Patong. In addition, the difference in lesion numbers between plants maintained at saturation and severely stressed plants was greater for UPLRi5 than C22 and Kanandong Patong with lesion number leveling at high stress levels. The partial resistance of UPLRi5 was reduced by drought stress. At soil saturation, there were more susceptible-type lesions on C22 than on Kanandong Patong and UPLRI5 whereas on severely stressed plants (S4), there were more susceptible-type lesions on UPLRi5 than C22 and Kanandong Patong. The increase in number of susceptible-type lesions with drought stress on different leaves also varied with cultivar. There was no significant curvature in the plot of ln(RIE+1) vs stress for leaves of C22. In contrast, on Kanandong Patong, there was a significant positive curvature in the plot of ln(RIE+1) for the fifth and sixth leaves, indicating that the increase in RIE was not a simple exponential function of stress presumably reflecting a stress threshold. On UPLRi5, the negative curvature presumably reflected the linear increase in lesion number with drought stress.

Generally, there were more resistant-type lesions on all leaves of all cultivars but, otherwise, drought stress and leaf position had similar effects on both types of lesions. One notable exception was that on UPLRi5 there were significantly more resistant-type lesions on the fifth than on the sixth leaf of plants maintained at soil moisture saturation. However, this difference gradually disappeared as drought stress increased.

Effect of drought stress on relative infection efficiency on leaf segments. The effects of drought stress on leaf elongation were similar for the two experiments in this series and only the results for the second are shown in Figure 2.1. Beginning 144 hours after water was withheld, leaf elongation decreased with increasing drought stress. Leaf development stage was highly variable within stress levels.

Both the number of segments with at least one lesion and relative infection efficiencies were correlated with leaf development stage in the first experiment. The number of segments per plant with susceptible-type lesions was negatively correlated with leaf development stage of the expanding leaf ($r=-0.318$, $P<0.001$) whereas there was no association between number of segments per plant with resistant-type lesions and leaf development stage. Susceptible-type lesion number decreased with leaf development stage on the second ($r=-0.321$, $P<0.001$) and third ($r=-0.262$, $P<0.001$) youngest segments. In contrast, resistant-type lesion number decreased on the second ($r=-0.1424$, $P<0.01$) and increased on the fourth ($r=0.1182$, $P<0.05$) youngest segments. Lesion number was independent of leaf development stage for all other leaf segments. There were no correlations between the number of infected segments or lesion number and leaf development stage in the second experiment. Because leaf development stage was correlated with the number of infected segments and lesion number in the first experiment only, it was used as a covariate in that experiment only.

Both the analysis of covariance in the first experiment and the analysis of variance in the second experiment showed that the number of infected segments increased with drought stress. There was an apparent stress threshold above which there was a sharp increase in number of segments with at least one susceptible-type lesion (Fig 2.2). The threshold level varied with experiment. In the first experiment, the mean number of segments with susceptible-type lesions increased significantly from 0.61 at low stress levels (S0-S6) to 1.63 at high stress (S7, [$P<0.001$]) whereas in the second experiment, mean number of lesions increased significantly from 3.57 at low stress levels (S0-S4) to 6.49 at high stress levels (S5-S7, [$P<0.001$]). Trends were similiar for the number of segments with resistant-type lesions in the first experiment whereas the number of segments with resistant-type lesions did not vary with stress in the second experiment.

The number of lesions varied with experiment, drought stress, segment number, and lesion type. Although there were fewer susceptible-type lesions in the first experiment (0.38 lesions/100 cm^2) than in the second experiment (11.82 lesions/100 cm^2), the effects of drought stress and segment position were generally similar in both experiments. There was a threshold stress level above which there was a dramatic increase in the number of both susceptible- and resistant-type lesions. The cumulative relative infection efficiency for both lesion types increased sharply at a stress level S7 in the first and at stress level S5 in second experiment. In the second experiment, leaf water potential (LWP) was an exponential function of the observed soil weight as a percentage of the soil weight at saturation ($R^2 = 0.95$).

$$(2.5)\quad \mathrm{LWP} = -\exp(6.433 - 0.0483\ \mathrm{PSWS})$$

Thus, when the cumulative relative infection efficiency was regressed against leaf water potential (eq 2.3), the best fit was attained by a piecewise function with a hinge at -12.05 bars (Fig. 2.3)

$$(2.6)\quad \mathrm{RIE}_{\mathrm{cum}}(Y) = \begin{cases} 33.28 - 4.24\cdot Y \text{ if } Y > -12.05 \text{ bars} \\ -1165.92 - 103.76\cdot Y \text{ if } Y < -12.05 \text{ bars} \end{cases}$$

The cumulative distribution of lesions over segment age varied with lesion type but all distributions were positively skewed (Figs. 2.4-2.5) as indicated by the positive p values (Table 2.4). Both susceptible- and resistant-type lesion numbers increased to a maximum on young segments and declined on older segments but in general the number of susceptible-type lesions peaked earlier and declined more rapidly than the resistant-type lesions as indicated by the larger p value (Table 2.4). In the first experiment, the maximum number of susceptible-type lesions was attained on 1.3-day-old segments of plants maintained at low to moderate stress levels (S0-S5) and on 1.3-2.3 day-old segments of plants at high stress levels (S6-S7) [Fig. 2.4A), whereas the maximum number of resistant-type lesions occurred on 2.3-3.3 day-old segments at low to moderate stress levels (S0-S5) and on 4.3 day-old segments at high stress levels (S6-S7) [Fig. 2.4B]. In the second experiment, maximum number of susceptible- and resistant-type lesions occurred on the 2.3- and 5.3-day-old segments, respectively, and decreased thereafter (Fig. 2.5). Overall, there were more resistant- than susceptible-type lesions in both experiments (Figs 2.4-2.5).

There was a sharp increase in susceptible- and resistant-type lesion numbers on most segments as stress levels increased beyond a threshold level. The threshold occurred at stress levels S7 and S5 in the first and second experiment, respectively. Lesion number increased further with increasing stress in the first experiment, but levelled off after plants attained a stress level of S6 in the second experiment.

The Richards model was adequate to model the cumulative distribution frequency of lesions on segments of various ages as a function of the stress levels. A number of values were tested to find the appropriate value for b and Q. The Q parameter was important because the rate and asymptote parameters converged only when the value of q was greater than zero. In each regression, p, b, S_O, and Q were held constant after initial estimates were determined. Model appropriateness was determined by R^2, residual plots, and normal plots. The value for m was always less than one, indicating that the distribution of lesions on leaf segments was positively skewed. All models explained a large proportion of the total variation due to treatments. The R^2 for the derivatives of the estimated Richards models was somewhat less than for the cumulative distribution function.

The intrinsic rate parameter of the Richards equation (= r in eq. 2.1 and k+RxSTRESS in eq. 2.2) reflects how the proportion of lesions on different segments changes with segment age. The values of R for susceptible-type lesions was significantly greater than zero in both experiments (Table 2.4), indicating that increased stress was accompanied by a decrease in the rate at which susceptible-type lesions declined on older segments. This phenomenom was particularily evident in the first experiment; unstressed segments older than 3.3 days had fewer lesions than stressed older segments. Because the decline of susceptible-type lesions with increasing segment age was less on stressed than unstressed plants, the number of segments with at least one susceptible-type lesion was greater on stressed than unstressed plants. In contrast, the intrinsic rate was constant in the equations describing the distribution of resistant-type lesions on leaf segments of increasing age and,thus, indicated that the number of resistant-type lesions on any particular segment was dependent only on the total number of resistant-type lesions (Fig 2.4B).

Effect of plant age and drought stress on relative infection efficiency. Lesion number was highly variable with the coefficient of variation on different leaves ranging from 40% on the (n-2)th leaf to 120% on the expanding leaf. There were more resistant- than susceptible-type lesions (Table 2.5).

Although there was considerable variation among plants within stress levels, mean development stage of the expanding leaf was reduced by stress. Thus, development stage of the youngest leaf was included in the analyses.

Lesion number varied with the number of leaves on the main tiller, development stage of the expanding leaf, leaf position relative to the expanding leaf, and drought stress. Lesion number decreased with the number of leaves on the tiller and increased with stress on all leaves (Table 2.5), with no interaction between these two independent variables. On all leaves, lesion number increased dramatically after a stress level of S6 was attained, once again indicating a stress threshold. The response to stress and development stage of the expanding leaf varied with leaf position (Table 5). On the expanding leaf, lesion number increased with stress at low stress levels (S0-S5) before increasing dramatically and leveling off at high stress levels (S6-S7). The decrease in lesion number with age depended on water stress. The decrease in lesion number with leaf age was steeper at high (S6-S7) than low (S0-S5) stress levels (Table 2.5, Fig. 2.6). On the second leaf, the pattern of lesion increase with stress was similiar to the expanding leaf, although the intercept was less reflecting fewer lesions. In contrast to expanding leaves, lesion number on the second leaf increased with age of the expanding leaf and the increase in lesion number with leaf age was more rapid at high than low stress levels. There were more lesions at the high stress (S6-S7) than low stress (S0-S5) levels, but no changes with stress within those levels. Furthermore, there was no effect of leaf development stage of the expanding leaf on lesion number (Table 2.5). Generally, trends were similiar for susceptible- and resistant-type lesions. However, coefficients generally were greater for resistant- than susceptible-type lesions.

2.4 Discussion

In the field, it is likely that blast is negatively correlated with plant rooting depth such that different cultivars growing in the same soil would experience different levels of drought stress. To avoid this possible confounding effect, relatively small pots were used to ensure that all cultivars were at nearly the same stress level at the time of inoculation. The pots were large enough, however, such that root growth was not restricted over the duration of plant growth of 30-35 days.

Although relative infection efficiencies increased with drought stress, the effect of drought stress varied with cultivar. Futhermore, although there was a pronounced stress threshold, the threshold varied with experiment (Fig. 2.2-2.5). Ambient conditions are likely to modify the effects of drought stress on the rice plant. For example, pre-inoculation conditions, in addition to drought stress, influence the susceptibility of the rice plant (Bonman et al. 1986, Yeh et al. 1989, Yoshino and Yamaguchi 1974). The enviromental conditions prior to and during the stress period are likely to be important in modifying the effects of stress on partial resistance. Thus, under field conditions, the effect of drought stress on epidemic development is likely to be very complex.

Susceptible-type lesions have a sporulating grey center with or without a brown to purple border whereas resistant-type lesions are dark brown without a grey center, small in size and nonsporulating (Ou 1985). In the present study, there were more resistant-type than susceptible-type lesions. However, estimates of resistant-type lesions are biased because the larger sized susceptible-type lesions may obscure some of the smaller resistant-type lesions, especially on young or stressed leaves where susceptible-type lesions are especially numerous. This phenomenom may partly explain why the maximum number of resistant-type lesions occurred on older segments than the maximum number of susceptible-type lesions. Nontheless, although there were more susceptible-type lesions on segments of stressed plants, the number of resistant-type lesions also increased with drought. The differences in the distribution of susceptible- and resistant-type lesions between expanding and fully expanded leaves (Tables 2.3, 2.5), and young and old segments (Figs. 2.4-2.5) might have been due to lower photosynthetic rates which could have possibly affected phenolic production, and lower silica and lignin content which might have restricted infection and lesion growth. Because of the longer leaf wetness duration required for infection on older leaves (Chapter 4), it is possible that there was some interaction such that lesions that would have been compatible on young leaves appeared incompatible on older leaves.

Currently, it is not known whether the distribution of lesions would be altered with leaf wetness, temperature and/or other factors. Recently, incompatible isolate-cultivar combinations have been used in testing soil conduciveness to rice blast (Singh et al. 1995ab). It would appear that the occcurrence of resistant-type lesions may be used as a criterion when selecting cultivars for resistance to infection and its interaction with drought stress. However, the use of resistant-type lesions is unlikely to be useful in screening for partial resistance if susceptible- and resistant-type lesions are differentially affected by factors such as segment age or drought stress (Fig. 2.4-2.5).

In previous work, only a few levels of water stress have been examined and hence only qualitative effects could be described. Lesion number increased with drought stress (El Refeai 1977, Gill and Bonman 1988) and decreased with plant and leaf age (Roumen et al. 1992, Kahn and Libby 1958, Volk et al. 1958, Torres 1986). Furthermore, duration of segment susceptibility was increased by drought stress and drought stress increased susceptibility regardless of plant age. By examining a wider range of stress levels, we were able to quantify the relationship between water stress and infection. In general, the relationship between water stress and infection was nonlinear and there appeared to be no single transformation that could linearize the relationship (Fig 2.4-2.6, Table 2.4-2.5). Perhaps more importantly, the stress threshold apparently varied with cultiivar and experiment, indicating that simulation of the effect of drought stress would be very difficult.

Although lesion number generally declined with leaf segment age, the lesion number was less on the youngest than slightly older segments (Fig. 2.4-2.5). The youngest segment was not fully unrolled at the time of inoculation and thus, may have received less inoculum per unit area, resulting in a reduction in lesion number.

A number of factors, including lignin and silica content, could play a role in determining infection efficiency in expanding and fully-expanded leaves. For example, leaf aging tends to be accompanied by increases of both silicon content and resistance to infection (Volk et al. 1968).

Drought stress can be quantified by measuring either soil water content or leaf water potential. The advantages of measuring soil water content is that it is easier, non-destructive, and strongly correlated with leaf water potential. Furthermore, leaf water potential can fluctuate greatly over short time periods in response to enviromental changes. For example, measurements taken under partial clouded and sunny conditions differed by as much as 5 bars. In contrast, soil water content is not as sensitive to rapid changes of the environment. Thus, water content of the soil profile might be the better predictor for blast. In simulators, soil water content could be modelled using initial soil water content, precipitation, pan evaporation, leaf area index and root length density as input variables (Herrera-Reyes and Penning de Vries 1990).

The Richards model was adequate for describing lesion distrubution over various aged segments. This, or similiar models, could be incorporated into a crop growth simulator to provide an estimate of the relative susceptibility of different aged leaves. In this way, it would be possible to simulate the distribution of lesions on leaves if different ages exposed to equal or different inoculum levels. Such an approach would require only a probability function such as equation 2.2.

The direct effects of stress on susceptibility to rice blast tend to be confounded with indirect effects that are mediated through a reduction in plant age. In previous experiments, where the goal was to examine the effects of stress on susceptibility, large numbers of seeds were used, with plants destined for the more severe stress treatments planted first so that all experimental units would be at approximately the same age at the time of inoculation (Gill and Bonman 1988). This approach is very time consuming. An alternative approach, to remove the leaf age effect statistically, seems feasible, given the linear relationship between transformed lesion numbers and both leaf development stage and number of leaves on the main tiller. If there was no interaction between stress and age, analysis of covariance, with plant age as the covariate, could be used to measure the direct effects of stress on susceptibility; if there were an interaction between stress and plant age, stress treatment effects could be compared at one or more reference ages. An analysis of covariance with leaf development stage as the covariate or an analysis of variance at more than one reference leaf development stage would be more informative in pathosystems where partial resistance varies with tissue age than in systems where partial resistance remains relatively constant with tissue age.

In summary, the effects of pre-inoculation drought stress increased plant susceptibility to infection. The rankings of three cultivars with a range in partial resistance were altered by pre-inoculation drought stress. The relative infection efficiency on the youngest leaf parts increased after subjecting seedlings at the fifth leaf stage to drought stress. In addition, whereas there was no or few infections on older leaf segmets of unstressed plants, the number of infections on older leaf segments increased with drought stress. These effects of drought stress were observed regardless of plant age.

Table 2.1. Analysis of variance for the effects of cultivar and drought stress on the number of susceptible- and resistant-type lesions on the main tiller. Relative infection efficiencies were transformed using ln(x+1).

		Susceptible-type Lesions			
Source	DF	SS	MS	F Value	Pr > F
Replicate	3	1.10	0.37		
Cultivar (V)	2	69.54	34.77	115.41	0.0001
Stress (S)	3	172.59	57.53	190.97	0.0001
VxS	6	51.97	8.56	28.75	0.0001
Error	33	9.94	0.30	1.05	0.3984
S. Error[#]	228	65.32	0.29		

		Resistant-type Lesions			
Source	DF	SS	MS	F Value	Pr > F
Replicate	3	5.41	1.80		
Cultivar (V)	2	191.70	96.85	192.67	0.0001
Stress (S)	3	98.86	32.95	66.24	0.0001
VxS	6	91.12	15.19	30.52	0.0001
Error	33	16.42	0.50	1.15	0.2695
S. Error[#]	228	98.37	0.43		

[$] Data transformed using ln(x+1), where x is the relative infection efficiency.

[#] Sampling error

Table 2.2 Analysis of variance for the effects of cultivar and drought stress on the number of susceptible- and resistant-type lesions on leaves of the main tiller. Relative infection efficiencies were transformed using ln(x+1).

Susceptible-type Lesions

Source	DF	SS	MS	F-ratio	Pr > F
Replicate	3	6.67	2.22		
Cultivar(V)	2	245.26	122.63	112.83[†]	.0001
Stress(S)	3	443.89	147.96	136.13	.0001
V x S	6	131.91	21.99	20.23	.0001
Error	33	25.99	0.79		
Leaf (L)	2	286.76	143.38	131.91	.0001[‡]
L x V	4	15.31	3.82	3.52	.0074
L x S	6	21.67	3.61	3.32	.0031
L x V x S	12	28.52	2.38	2.19	.0110
Error	72	60.93	0.85		
S. Error[±]	684	743.47	1.09		

Resistant-type Lesions

Source	DF	SS	MS	F-ratio	Pr > F
Replicate	3	8.68	2.89		
Cultivar(V)	2	394.40	197.20	120.65	.0001
Stress(S)	3	338.85	112.95	69.11	.0001
V x S	6	295.70	49.28	30.15	.0001
ERROR	33	43.29	1.31		
Leaf (L)	2	314.70	157.35	96.27	.0001[‡]
L x V	4	43.58	10.89	6.67	.0001
L x S	6	65.39	10.90	6.67	.0001
L x V x S	12	65.17	5.43	3.32	.0001
Error	72	116.45	1.62		
S. Error[±]	684	1117.95	1.63		

[†] F-ratios were calculated using the sampling error since error terms were not significant.

[‡] Greenhouse-Geiser probabilities.

[±] Sampling error.

Table 2.3. Number of susceptible- and resistant-type lesions/100 cm^2 on different leaves and the main tiller of three cultivars subjected to drought stress and the t-values for polynomial contrasts for stress on different leaves. Relative infection efficiencies were transformed using ln(x+1).

		Susceptible-type			Resistant-type		
Stress	Leaf	C22	KP	UPLRi5	C22	KP	UPLRI5
1	6	27.77†	4.17	3.37	26.79	4.19	31.53
	5	28.56	5.84	4.20	56.15	7.52	169.69
	4	8.10	1.40	1.40	13.37	1.58	5.21
	MT	25.53	5.21	4.53	42.95	6.62	95.58
2	6	26.36	4.42	36.78	77.17	4.27	172.43
	5	24.29	1.91	14.78	65.43	2.16	140.19
	4	10.95	1.59	4.73	6.90	2.16	30.11
	MT	25.53	3.63	26.84	72.24	4.62	181.27
3	6	65.24	31.37	44.43	103.75	36.93	189.24
	5	35.59	7.35	57.51	51.37	27.61	190.76
	4	11.46	5.95	8.87	31.47	26.02	31.63
	MT	45.15	18.36	55.70	93.69	44.26	221.41
4	6	72.31	74.51	85.03	76.48	165.84	135.10
	5	55.53	20.43	48.13	166.00	64.59	43.03
	4	22.29	9.45	16.66	24.22	37.98	24.88
	MT	62.80	45.15	89.12	126.47	122.73	132.95
Linear							
leaf 6		5.13‡	14.83	13.74	3.82	15.12	5.06
leaf 5		5.40	5.36	9.02	2.50	7.68	-3.23
leaf 4		3.09	7.41	8.36	2.74	10.24	4.01
leaf 5-leaf 6		0.90	-3.25	-7.18	-0.21	2.05	-4.01
leaf 4-leaf 5		1.16	1.02	-0.33	0.12	1.28	3.62
Quadratic							
leaf 6		0.28	1.95	-4.64	-2.91	3.18	-4.44
leaf 5		-0.97	4.89	-3.34	1.89	3.93	-2.45
leaf 4		0.79	0.86	-1.36	0.73	0.14	-3.78
leaf 5-leaf 6		1.22	-4.78	0.38	-2.36	0.62	-0.74
leaf 4-leaf 5		0.39	2.92	-0.99	-0.56	-1.89	-0.66

† Entries in original scale

‡ t-statistic based on orthogonal contrasts on transformed data. Contrasts are significant at P= 0.05 and P=0.01 if the absolute value of the statistic exceeds 1.98 and 2.62, respectively.

Table 2.4. Parameter estimates for the function determining cumulative relative infection efficiency[1] over segments of increasing age in two experiments. Values for susceptible-type and resistant-type lesions are shown separately.

		Susceptible-type		Resistant-type	
Exp	Parameter	Estimate	se	Estimate	se.
1	c1	1.26	0.17	1.46	0.19
	d1	0.56	0.06	0.79	0.07
	c2	-75.72	3.20	95.11	10.50
	d2	12.61	0.51	17.29	1.80
	S0	5.5	-	5.5	-
	p	2.22	1.17	0.24	0.28
	k	5.45	1.82	0.60	0.09
	R	0.77	0.26	0.04	0.02
	Q	1.00	-	1.00	-
	R^2 [†]	0.93		0.98	
	Nc[‡]	0.98		0.99	
Dx/Dy	R^2[±]	0.79		0.78	
2	c1	34.11	3.81	222.89	23.51
	d1	10.41	1.55	25.03	4.21
	c2	-1105.39	96.27	-883.91	115.01
	d2	256.08	19.47	284.45	31.00
	S0	4.5	-	4.5	-
	p	1.14	0.11	0.47	0.05
	k	0.48	0.14	0.17	0.03
	R	0.05	0.02	0.0	-
	Q	1.00	-	1.29	-
Cdf	R^2	0.99		0.99	
	Nc	0.94		0.98	
Dx/Dy	R^2	0.78		0.89	

[1] Mean values of segment RIE for each level of drought stress were used.

[†] Coefficient of determination for the cumulative RIE function

[‡] Correlation between observed residuals and those expected under the normality assumption.

[±] Coefficient of determination for derivative of the cumulative function

Table 2.5. Parameter estimates for relative infection efficiency as a function of position and development stage of the expanding (nth) leaf (Age) and drought stress (S0 = Saturation, S7 = Leaf roll 5), determined by stepwise regression. Variables were entered at P=0.050. A "ns" indicates the coefficient was not significant.

	Leaf Position					
	n		n - 1		n - 2	
	Estimate	se	Estimate	se	Estimate	se
	Susceptible-type lesions					
Leaf no.[†]	-0.33	0.05	-0.21	0.04	-0.13	0.04
Intercept 1[‡]	4.06	0.30	1.36	0.23	1.31	0.20
Stress	0.31	0.04	0.08	0.03	ns	-
Age	-1.13	0.06	0.54	0.16	ns	-
Intercept 2[±]	7.01	0.28	2.16	0.22	1.69	0.09
Stress	ns	-	ns	-	ns	-
Age	-3.20	0.38	1.22	0.29	ns	-
R^2[⸸]	0.35		0.24		0.05	
	Resistant-type Lesions					
Leaf no.[†]	-0.15	0.06	-0.36	0.05	-0.29	0.05
Intercept 1[‡]	4.77	0.35	2.65	0.33	2.99	0.29
Stress	0.29	0.04	0.18	0.04	ns	-
Age	-3.35	0.08	1.19	0.23	ns	-
Intercept 2[±]	7.16	0.33	3.51	0.31	3.80	0.13
Stress	ns	-	ns	-	ns	-
Age	-4.84	0.45	2.127	0.42	ns	-
R^2[⸸]	0.32		0.19		0.10	

[†] The leaf number of the expanding (nth) leaf

[‡] Intercept 1 corresponds to ln(RIE) predicted at stress levels S0.

[±] Intercept 1 corresponds to ln(RIE) predicted at stress levels S6.

[⸸] Coefficient of determination

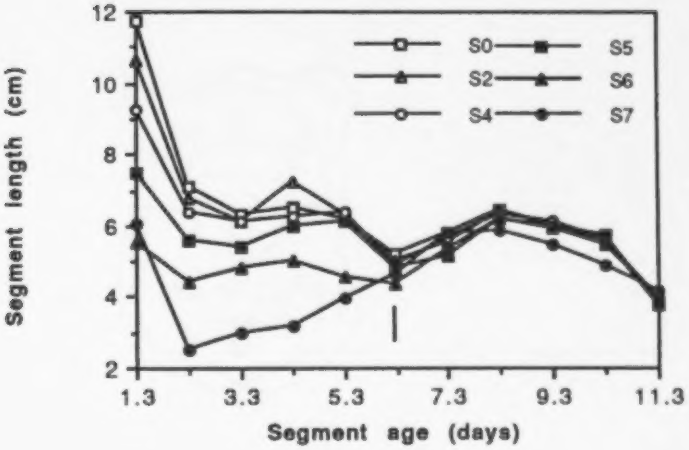


Fig. 2.1. The effect of water stress (S0 = Saturation, S7 = Leaf roll 5) on the length of leaf segments of various ages at the time of inoculation. The stress main effect and the interaction of stress with segment interaction were both significant at $P < 0.01$. The $LSD_{0.05}$ is shown by the bar.

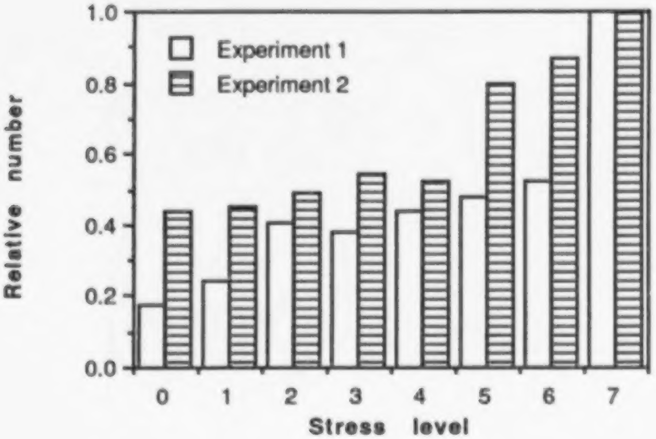


Fig. 2.2. The relative number of leaf segments with at least one susceptible-type lesion on C22 at different stress levels (S0 = Saturation, S7 = Leaf roll 5) in two experiments. The number of segments is relative to the numbers at stress level S7 (1.63 and 7.30 in the first and second experiment, respectively). The effect of stress was significant at $P < 0.05$ and 0.01 in the first and second experiment, respectively.

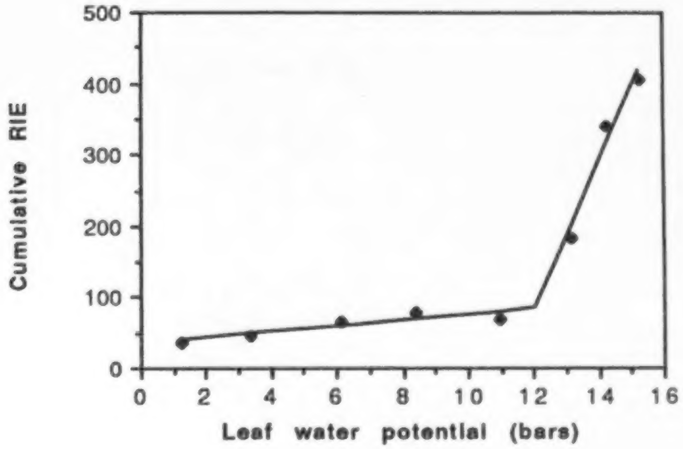


Fig. 2.3. Increase in cumalative relative infection efficiency with leaf water potential. The slopes of the two lines (piece-wise regression) were significantly different at $P < 0.001$.

Fig. 2.4. Lesion number/100cm^2 on different leaf segments of C22 subjected to different levels of drought stress (S0 = Saturation, S7 = Leaf roll 5) in the first experiment. (A) Susceptible-type lesions. Stress, segment, age and segment with age interaction effects were all significant at $P < 0.001$. (B) Resistant-type lesions. Stress, segment, age and segment with age interaction effects were all significant at $P < 0.01$, 0.05, 0.05 and 0.05, respectively. The prediction lines are the first order differences of eq. 2.2 over consecutive segments for each stress level. Estimates of model parameters are listed in Table 2.4 (Experiment 1).

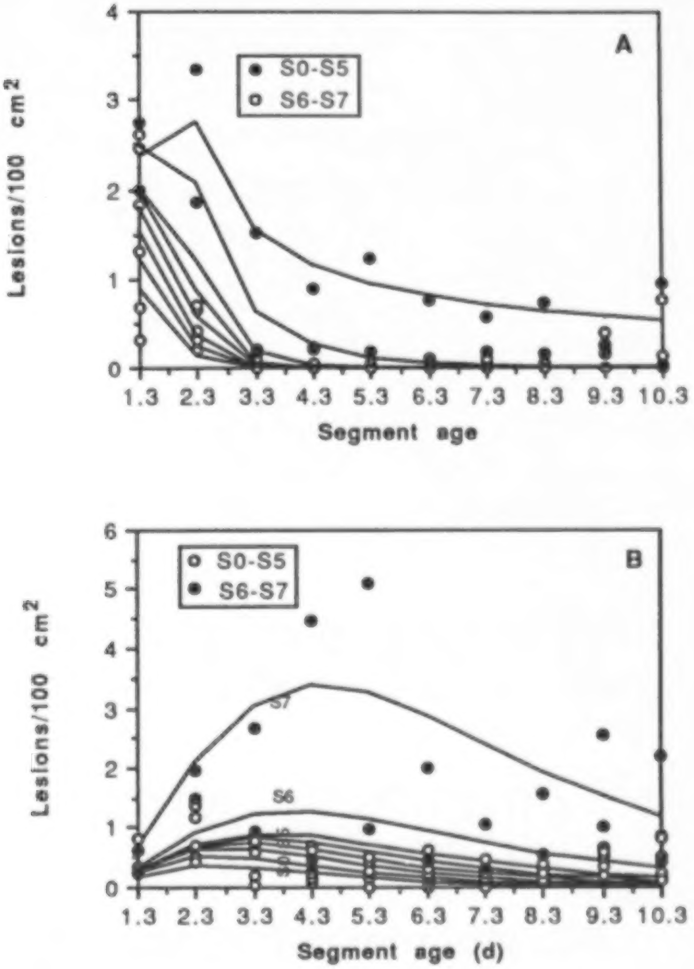
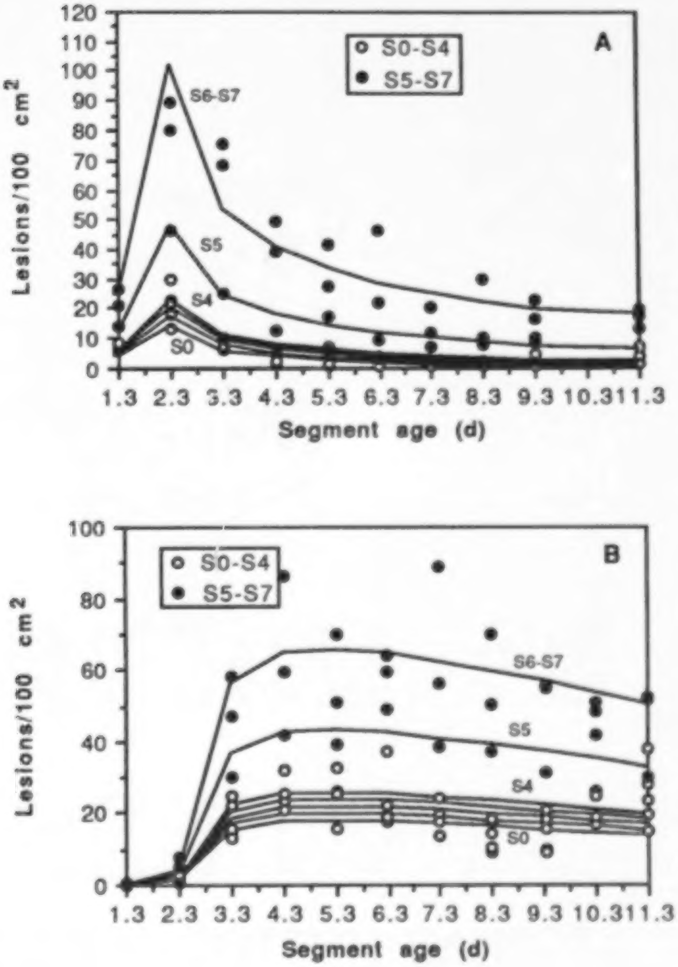

A
● S0-S5
○ S6-S7
Lesions/100 cm²
0
1
2
3
4
1.3
2.3
3.3
4.3
5.3
6.3
7.3
8.3
9.3
10.3
Segment age
B
○ S0-S5
● S6-S7
S7
S6
Lesions/100 cm²
0
1
2
3
4
5
6
1.3
2.3
3.3
4.3
5.3
6.3
7.3
8.3
9.3
10.3
Segment age (d)

Fig. 2.5. Lesion number/ 100 cm^2 on different leaf segments of C22 subjected to different levels of drought stress (S0 = Saturation, S7 = Leaf roll 5) in the second experiment. (A) Susceptible-type lesions. Stress, segment and the stress with segment interaction were significant at $P < 0.001$, 0.05 and 0.05, respectively. (B) Resistant-type lesions. Stress, segment and the stress with segment interaction were all significant at $P < 0.001$. The prediction lines are the first order differences of eq. 2.2 over consecutive segments for each stress level. Estimates of model parameters are listed in Table 2.4 (Experiment 2).


A
S0-S4
S5-S7
S6-S7
S5
S4
S0
Lesions/100 cm^2
0
10
20
30
40
50
60
70
80
90
100
110
120
1.3
2.3
3.3
4.3
5.3
6.3
7.3
8.3
9.3
10.3
11.3
Segment age (d)
B
S0-S4
S5-S7
S6-S7
S5
S4
S0
Lesions/100 cm^2
0
20
40
60
80
100
1.3
2.3
3.3
4.3
5.3
6.3
7.3
8.3
9.3
10.3
11.3
Segment age (d)

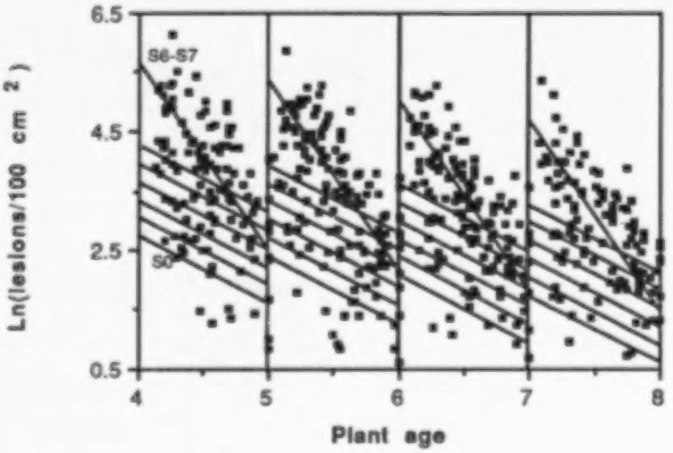


Fig. 2.6. The number of susceptible-type lesions on the expanding leaf of plants at the 5, 6, 7 and 8 leaf stage. Plant age was determined by the leaf number of the (n-1)th leaf and the proportion of the nth leaf emerged at inoculation. Stress, and stress with leaf age interaction effects were significant at $P < 0.001$ and 0.05, respectively. The solid lines are those predicted by the regression model in Table 2.5 for the expanding leaf (leaf position n).

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Chapter 3. Effect of pre-inoculation drought stress on infection, incubation period, lesion size and sporulation of *Magnaporthe grisea* on rice.

3.1 Introduction

One of the major contraints for rice production in rainfed lowland and rainfed upland agroecosystems is water availability (De Datta 1981) and yields may be severely restricted if drought stress occurs at panicle initiation. In addition, rice blast, caused by *Magnaporthe grisea* (Herbert) Barr, is exacerbated in the rainfed lowlands, and is even worse in rainfed uplands, because drought stress increases host susceptibility (Gill and Bonman 1988, Bonman et al. 1988, El Refaei 1978).

Disease progress depends on a number of epidemiological parameters including infection efficiency, latency and sporulation. These parameters may be modified by host resistance and environmental conditions. For example, infection of rice by *Magnaporthe grisea* is influenced by tissue age (Kahn and Libby 1958, Roumen 1993), with the time required for leaves to become resistant to infection varying with cultivar (Roumen 1993). One effect of pre-inoculation drought stress was to increase susceptibility to infection (Gill and Bonman 1988). In addition, drought stress offset some ot the increased resistance associated with aging, by prolonging the susceptibility of the older leaf parts. (Chapter 2). Disease severity was lower on irrigated than drought stressed plots, even though the leaf wetness duration was greater on the former (Bonman et al. 1988). It could be hypothesized, therefore, that drought stress reduces the minimum period of leaf wetness required for infection. Furthermore, given that plots of infection vs. time tend to be sigmoidal, drought stress could presumably affect either the value of the asymptote or the rate at which the asymptote is approached.

Pre-inoculation drought stress increased lesion size and sporulation per unit area but not latent period (Gill and Bonman 1988, Kato and Kozaka 1975, El Refeai 1977). However, in all of these experiments isolated lesions were used to determine lesion expansion and sporulation. This simplied approach may have produuced results unrepresentative of those expected under field conditions. Different results might have been obtained if multiple lesions were permitted to interact. To date, there has been no study on the effect of lesion number on latent period, lesion size and sporulation in the blast pathosystem. However, in the case of the *Hordeum vulgare-Puccinia hordei* pathosystem, latent period varied with lesion number (Teng and Close 1978).

In this paper, we examined the interaction between drought stress and leaf position on lesion number over different periods of leaf wetness. The effects of drought stress on lesion size, latent period and sporulation were reexamined to account for lesion number.

3.2 Methods and Materials

General methods. Except as noted, the following general methods were employed in all experiments. Experiments were conducted in the greenhouse at the International Rice Research Institute (IRRI), Los Banos, Philippines. There was no artificial lighting and temperatures ranged from 22C to 38C during the experimental period.

Plants were grown in plastic pots without drainage holes. Maahas silty clay loam soil (Typic Hapludoll) was obtained from the upland site at IRRI, air dried and sieved to remove large clumps. An equal weight of soil was added to each pot. In some experiments, the following procedure was used to prevent puddling of water and alteration of soil structure. One cm of soil was added to each pot. A plastic pipe, 20 cm in length and1.0 cm in diameter, was placed in the center of the pot and the remaining soil was placed around the pipe; the soil was saturated by adding water through the pipe.

Initially, each pot was planted with six seeds which had been pre-germinated on filter paper in petri dishes for 48 hours in the dark at 25 C. In later experiments, 8-12 seeds were planted into each pot. After 7 days, the number of plants were thinned to 5-8 seedlings of uniform age. Plants were fertilized with ammonium sulphate (50 kg/ha) in solution at the time of seeding.

Soil moisture levels were determined gravimetrically. To determine the pot weight at which severe drought stress occurred, an additional four pots were prepared for each experiment. Water was withheld from two of these four pots until severe leaf rolling occurred (5 on the IRRI leaf roll scale [O'toole and Cruz 1979]). Leaf rolling was reversible when plants were rewatered. Using the pot weights at saturation and severe drought stress as end points, pot weights for the remaining levels of water stress were determined by interpolation.

Soil was maintained near saturation for the first 15 days after seeding for all treatments by adding water, twice daily, to reestablish saturation. Thereafter, water was withheld for all treatments except the saturated soil treatment. Intermediate levels of water stress were produced by allowing the soil to dry until pot weights reached the level of the desired stress treatment. Subsequently, pots were weighed twice daily and water was added to maintain the desired water stress. The highest level of water stress was produced by withholding water until severe leaf rolling (leaf roll 5) occurred, 7-12 days later. At this point, all pots in the experiment were watered to within 25 g of saturation, and then an additional 25 ml of ammonium sulphate (25 kg/ha) solution was added. Immediately after this final watering, a fine mark was placed on the expanding leaf at the point where it was emerging from the sheath. This was done to enable determination of plant age at the time of inoculation. Plants were inoculated in the late afternoon. Following inoculation, soil saturation was maintained.

Inoculum was prepared following the methods of Mackill and Bonman (1985). Isolates were obtained from the Entomology and Plant Patholgy Division, IRRI. Colonized sorghum seed was placed on prune agar in a test tube and incubated for 7 days. Ten mL of distilled water was then placed in the test tube and the colony was scraped with a bacterial transfer loop. The resulting suspension of conidia and mycelial fragments was poured onto glass petri dishes containing oatmeal agar. The plates were incubated for 7 days at 27 C. Then the mycelial mat was scraped with a rubber policeman and the plates, with the lid removed, were placed in a cabinet with a fluorescent light for 3 days. These plates were used for inoculum or stored in a cold room for a period of not more than 3 days. The period of storage was necessary because of uncertainty in predicting when leaf roll would occur. On the day of inoculation, distilled water was added to the petri dish, the mycelial mat scraped with a sterilized rubber policeman or a glass slide, and the suspension filtered through four layers of cheese cloth. Conidia were counted with a haemocytometer and the concentration of the suspension was adjusted to 1 x 10^5 per ml. An equal volume of a 1% gelatin solution was added to obtain a final concentration of 0.5 x10^5 conidia per ml in 0.5% gelatin.

The plants, grouped by blocks, were sprayed, until runoff, with the conidial suspension. They were then placed into a dew chamber at 25 C with one or two blocks per chamber depending on the number of treatments. Plants were removed from the dew chamber 16 hours later and transferred to an incubation room maintained at high humidity and 25-30 C. Plants were assessed for disease 5-7 days later.

Six to seven days after inoculation, the youngest three leaves were removed from the plant and placed onto paper using clear tape as the mounting material. The entire leaf was placed onto the tape prior to mounting onto paper. Sporulating lesions were counted on each leaf and leaf dimensions were taken to estimate leaf area.

Effect of drought stress, leaf position and leaf wetness duration on infection efficiency. In the first experiment, there were twelve replicates, one cultivar (C22), two stress levels and four leaf wetness durations (12-21 h). The plants were at the eighth leaf stage when inoculated.

In the second experiment, there were two replicates, three cultivars (C22, OS-6, and UPLRi-5), one isolate (V850256) which was compatible on all cultivars, three stress levels and six leaf wetness durations (9-36 h). The plants at the fifth leaf stage when inoculated.

In the third experiment, the same isolate and cultivar as in the first were used. There were three replications. In contrast to the previous experiments, an upland clay soil (Orthoxic Palehumult) from Cavinti, Laguna, Philippines, was used. Water was added through a PVC pipe, in contrast to the first two experiments in which water was added directly to the soil. There were an increased number of stress levels (four) and leaf wetness durations (nine). The plants also were younger, at the fifth leaf stage, when inoculated.

In all cases, plants were dried after being removed from the dew chamber and placed in an incubation room. Seven days after inoculation, the youngest three leaves at the time of inoculation were stripped from the main tiller and placed onto paper with clear tape. For each treatment, the minimum leaf wetness duration required for infection was defined as the first time at which at least one lesion was seen for that treatment in any replicate.

For each experiment, leaf positions were treated as repeated measures. In all experiments, except the first, trends analysis was used to evaluate the effect of different levels of stress on lesion number. It was also used to evaluate the rate of increase of lesion number with increasing leaf wetness duration over that period in which the increased appeared to be linear. There were too few stress levels to do this in the first experiment.

Conceptually, stress could affect the relationship between leaf wetness duration and lesion number in two ways: by affecting the asymptotic level of lesion number or by the affecting the rate at which the asymptote was approached. To explore these possibilities, the effect of soil moisture on the parameters of a Richards model was determined for the second and third experiments. The model consisted of two functions, the first predicted the asymptote and the second used the Richards model to estimate the proportion of the asymptotic number of lesions arising from infections at a given leaf wetness duration. The model was fit separately by experiment, variety and leaf position. A constant value for the shape parameter was used for all leaf position and variety treatments in a given experiment. This avoided problems of model singularity and permitted asymptotes and rates for the different treatments to be compared on the basis of a common shape parameter. This shape parameter was selected on the basis of R^2 and residual pattern.

An iterative approach was used to determine the model parameters in the second experiment which had three stress levels. First, the asymptote and rate parameters were estimated for two highest stress levels. If the parameter estimates for these two stress levels were not significantly different, these parameters were held to be equal in the next iteration. However, if either or both estimates for asymptote and the rate parameter were significant, then in the next iteration, unique parameter estimates were made for all stress levels. In the second iteration, the parameters for the water stressed and saturated treatments were estimated.

In the third experiment, the data for the first three stress levels (= no leaf roll) were combined because there were no significant differences in relative infection efficiency among these levels. Parameter estimates for the leaf roll and no leaf roll treatments were estimated.

Effect of drought stress and leaf segment on initial lesion size. In this experiment, only the susceptible cultivar C22 and the isolate P06-6 was used. Twelve seeds/pot were planted into 80 pots which were thinned to 8 seedlings seven days later. Eight stress treatment levels were applied as described above. Pots were arranged into 10 blocks in a randomized complete block design.

Weights for all pots were recorded for the duration of stress period. At the beginning of the stress period and at each of the twice daily weighings thereafter, all plants in a pot were marked at the base of the expanding leaf. This demarcated the leaf growth that occurred during the stress period into leaf segments and provided a measure of leaf elongation.

Beginning one day after the appearance of susceptible-type lesions, the marked leaves were removed from each plant. Maximum length and maximum width of each leaf, the distance from the base to the point of the maximum width, the distance from the base of the leaf to each felt pen mark and the width at each mark were recorded. Segments at the tip of the nth leaf included the base of the (n-1)th leaf. Susceptible-type lesions were counted for each segment .

Lesion areas were estimated by comparing the lesion with a computer generated chart containing lesions of different areas and four different length:width ratios per lesion area (Roumen, 1993).

Effect of drought stress and leaf segment on incubation period and sporulation. Two experiments were conducted. There were three levels of soil moisture in the first experiment and five levels of soil moisture in the second experiment. In addition, a number of pots were planted for each treatment, so that plants could be sampled destructively over time. Both experiments consisted of three replicates arranged in a randomized complete block design. Using a fine felt pen, marks were placed on the expanding leaf at the beginning and the end of the stress periods in both experiments. In the second experiment, 48 and 96 hours after imposing water stress, marks were also placed on the expanding leaf to demarcate three leaf segments; inoculation occurred 104 hours after water stress began. To determine the incubation period, defined as the time at which 50 per cent of the lesions formed on a particular leaf, susceptible type lesions on each leaf or segment were counted beginning 72 hours after inoculation. Subsequently, counts were made 88, 96, 112, 120, and 136 hours after inoculation. No increase in lesion number was observed during the last 16 hours in either experiment.

Because of poor light conditions in the incubation room, plants were moved outdoors in the morning and were placed inside after 8 hours or prior to the onset of rain. In the first experiment, plants were first moved outdoors on the seventh day after inoculation whereas, in the second experiment, plants were moved out doors four days after inoculation. In the incubation room, leaves were kept dry at all times, although the humidity was allowed to increase.

Sporulation was measured beginning 4 days after inoculation and on 14 and 12 occasions over the next 19 and 24 days for the first and second experiment, respectively. In the first experiment, leaf segments, approximately 5 cm in length, were excised. In the second experiment, the youngest leaf segments, or the youngest two leaf segments if there were lesions on the second youngest leaf segment, were excised. Each segment was wiped with a wet cotton swab to remove spores on the surface and placed in a test tube with 0.1 ml distilled water. The test tube was closed with a cotton swab and placed in a test tube rack. The rack was wrapped with a moist cotton bag and placed in an open plastic tray with approximately 0.5 cm water in the bottom of the tray. A second inverted tray was placed over the first. The trays were incubated in the incubation room with overhead sprinklers for 16 hours. After incubation, an additional 0.4 ml of a 10% ethanol solution was added to the test tube and shaken vigorously with a vortex mixer such that the entire leaf was washed. The leaf was removed from the test test tube, taking care to remove excess water, and mounted onto paper. Two 0.01 ml aliquots of the spore suspension were placed on water agar, and allowed to dry for 2 minutes before all spores in the drop were counted.

Length and width of the excised leaf segment were measured. Lesion size and sporulating area were estimated using the lesion size chart and percentage senescent area on each segment was estimated visually.

Incubation period was estimated by linear interpolation using the end points of the time interval in which 50% of total number of lesions occurred. These data were square root transformed and subjected to analysis of variance. Analysis of variance was conducted on the sporulation data which were transformed using ln(x+1), where x is the mean spores/10 mm^2 grey area. Partial correlations between spore number per 10 mm^2 grey area, lesion number, sporulating area and senescent leaf area after adjustment for stress and segment effects, were calculated.

Effect of lesion density and leaf age on incubation period. Soil (Typic Hapludoll) was obtained from the upland site at the International Rice Research Institute, Los Banos, Philippines. After screening, the soil was placed into twelve plastic trays (30 x 24 x 12 cm^3). Twenty-five seeds of variety C22 were seeded in 6 rows/tray. Plants were grown under aerobic conditions by placing the trays in larger pans filled with 3 cm of water which was replenished after it had completely evaporated. The trays were fertilized with a ammonium sulphate solution at a rate of 50 kg N/ha (1.82 g/tray) after the seedlings emerged.

At 21 days after seeding, the expanding leaf on the main tiller of the plants from two rows per tray were marked at the point of exsertion with a fine felt pen. The trays were divided into three groups of four trays for inoculation at three inoculation densities (0.5, 1 and 2 X 10^5 spores/ml) using isolate P06-6. Following inoculation, trays were placed into dew chambers for 16 hours.

Beginning 72 hours after inoculation, susceptible-type lesions were counted on each marked leaf from the mark to the leaf tip. Subsequent counts were conducted 88, 96, 112, 120, 136 and 144 hours after inoculation. Leaf length, leaf width and the distance from the leaf collar to the mark were recorded on the last counting. Each leaf was considered to be in one of three age classes (0.00 - 0.33, 0.33 - 0.66, or 0.66 - 1.00) depending on the ratio of their length at inoculation to their final length.

Square root transformed values of the incubation period were subjected to analysis of covariance with inoculum level and leaf age as the independent variable and covariate, respectively. The correlation between transformed incubation period and transformed relative infection efficiency (ln(x+1)) were calculated based on the error sum of squares and cross-products matrix of the multivariate analysis of covariance that used these factors as these the dependent variables and leaf age and inoculum as the independent variables.

3.3 Results

Effect of drought stress and leaf wetness duration on infection efficiency. There were three experiments in this series. In the first experiment, which used older plants, there were fewer lesions than in the other two experiments which used younger plants.

In the first experiment, the minimum leaf wetness duration for lesion appearance varied with leaf position, stress and their interaction (Fig. 3.1). For example, on the eighth leaf, lesions first appeared after 12 hours, regardless of whether plants were stressed. In contrast, on the sixth leaf, water stress decreased the minimum leaf wetness duration required for infection. The first lesions appeared on stressed plants after 12 hours of leaf wetness, but on unstressed plants after 21 hours of leaf wetness. In the second experiment, the mininum leaf wetness duration varied with cultivar, leaf and stress (Fig. 3.2). On the fourth leaf of C22, the first lesions appeared on unstressed plants after 15 hours of leaf wetness whereas on stressed plants lesions first appeared earlier, after 9-12 hours of leaf wetness. The minimum leaf wetness for infection on the fifth leaf of C22 was 9 hours regardless of stress level. On UPLRI5, the minimum leaf wetness duration was 12 hours for leaves of both stressed and unstressed plants. In the third experiment, minimum leaf wetness duration for lesion appearance was shorter for the expanding fifth leaf (6.5 hours) than the older third and fourth leaves (8 hours). On the expanding leaf, there were no significant effects of drought stress on minimum leaf wetness duration, although infection occurred on 6 and 23 percent of the leaves of unstressed and stressed plants, respectively. After 8 hours of leaf wetness, the effect of stress on the proportion of infected leaves at lower positions was small. On the fourth leaf, infection occurred on 29 and 35 percent of the leaves of unstressed and stressed plants, respectively, whereas on the third leaf, 14 and 13 percent of the leaves of unstressed and unstressed plants, respectively, were infected.

In the first experiment, lesion numbers increased linearly with leaf wetness duration and were still increasing on the 7th and 8th leaves at the final assessment time, 21 hours after inoculation (Fig 3.1). Slopes of lesion number on leaves 8 and 6 of stressed plants were significantly greater than slopes of unstressed plants

In the second and third experiments, several levels of stress were applied in an attempt to quantify the relationship between water stress and lesion number, In neither experiment, however, was there a linear effect of water stress on lesion number, although there were significantly more lesions at leaf roll than at the average of the lower stress levels.

In the second experiment, lesion number increased rapidly after 12 hours and leveled off after 21 hours of leaf wetness. There were cultivar differences in mean number of susceptible lesions with numbers greater on C22 than UPLRi5 (Fig. 3.2) and no lesions on cultivar OS6 which was excluded from further analyses. Because of a significant cultivar x leaf position interaction, subsequent analyses were done separately by cultivar. On C22, there were more lesions on leaf five than leaf four, more lesions on leaf-rolled than less stressed plants, and no stress x leaf position interaction. In contrast, on UPLRi5, water stress increased lesion number, but the effect was greater on leaf four than on leaf five such that there was no consistent effect of leaf position (Fig. 3.2). Trends analysis, applied to lesions formed at 9-21 hours for each leaf separately, revealed a significant stress x leaf wetness duration interaction only on leaf four. On this leaf, the linear rate of increase with leaf wetness duration was significantly greater for the average of plants subjected to drought than plants at saturation ($P< 0.05$ and $P<0.001$ for C22 and UPLRi5, respectively).

In the third experiment, the number of lesions increased rapidly after 8 hours and began to level off 16-20 hours after inoculation (Fig. 3.3). Although drought stress, leaf wetness period and leaf position all had a significant effect on lesion number, the only significant interactions were the leaf position with leaf wetness duration and stress with leaf position effects. Lesion number on tightly-rolled leaves was greater than the average of less stressed plants. Trends analysis, applied to number of lesions formed at 6.5-12.5 hours of leaf wetness for each leaf separately, indicated a significant leaf position x stress x leaf wetness duration interaction. Lesion number increased linearly and the rate of infection on the fourth and fifth leaves was significantly greater for leaf rolled than the average of less stressed plants ($P<0.05$). Numbers and trends of lesions were similar on the third leaf of stressed and unstressed plants.

The effect of the water stress x leaf position interactions on the linear trend of increasing infection with increasing leaf wetness duration could have occurred from the effects on either a) the asymptotic level of infection or b) the rate at which the asymptote was approached. To explore these two possibilities, nonlinear regression was used to estimate the asymptote and rate parameter of a Richards model fit to the plot of mean-square-root-number of lesions versus leaf wetness duration. Rate parameters were relatively insensitive to the selected shape parameters which indicated that all curves were skewed to the right. In the second experiment, the rate and asymptote parameters varied with cultivar, stress, and leaf position. For example, water stress had no effect on the rate parameter on the fifth leaf whereas on the fourth leaf, rates were greater for stressed plants than for plants at saturation (Table 3.1). On the fifth leaves, the asymptotes were greater on stressed plants than unstressed plants and asymptotes were similar for increasing levels of stress. In contrast, the asymptote level of infection increased with stress on the fourth leaf. On unstressed plants, the rate and asymptote were significantly less on the fourth than fifth leaf of both cultivars. In the third experiment, the rate parameters were not significantly different between stressed and unstressed plants. The rate increased with leaf position, but only rates for the third and fifth leaf were significantly different (Table 3.2). The asymptotes on the fourth and fifth leaf were significantly greater on severely leaf rolled than unstressed plants. There was no effect of stress on the asymptote for the third leaf.

Effect of drought stress and leaf segment on initial lesion size. Results were similar for both experiments; the scatter plots for mean lesion size in the second experiment are shown in Fig. 3.4 Average lesion size decreased with segment age in both experiments although the linear effect accounted for only 6.7% of observed variation. There was no effect of pre-inoculation drought stress on lesion size nor an interaction of drought stress with segment position. There was a significant but small partial correlation ($r=0.096$, $P<0.05$) between lesion density and lesion size following adjustment for leaf segment and drought stress.

Effect of lesion density and leaf age on incubation period. Although seeding was done once, a range of plant ages (3.1-4.3 [leaf number + proportion]) was obtained and relative infection efficiency varied with plant age (Table 3.3). No significant differences were observed in relative infection efficiency with inoculum dose perhaps because of high variability, even within leaf age class. Incubation period was shorter on leaves in the youngest leaf age class (0.00-0.33) than older leaf age classes at the same leaf position. However, this difference did not exceed 6.4 hours. Furthermore, on leaves that had grown less than one third of the final leaf length, there was no relationship between leaf position and incubation period. There was a weak negative correlation between incubation period and lesion density ($r=-0.134$, $P<0.05$). The estimated regression equation for incubation as a function of both age and lesion number was $IP^{0.5} = 10.24+0.39\ AGE-0.18\ LNRIE)$ [$R^2 = 0.20$], where AGE is the proportion of the length of leaf at inoculation to its final length and LNRIE is the logarithmic transformed lesion density.

Effect of drought stress and leaf segment on incubation period and sporulation. In this series, two experiments were performed. Incubation periods were highly variable, with an average and range of 95.4 and 72 -116 h, respectively, across experiments In the first experiment there was a significant ($P<0.05$) but small effect of pre-inoculation stress on incubation period with an estimated difference of 5 hours between leaf rolled and water saturated plants. In the second experiment, stress, segment position and the interaction of stress with segment position, were not significant. The largest estimated differences among means of moisture treatments and segment position were 3.7 and 4.5 hours, respectively.

Sporulation, measured as spore number per unit area, increased one day after lesion appearance (Fig. 3.5). Sporulation varied with experiment (Fig. 3.5). In the first experiment, spore numbers decreased after the initial observation and then increased to a maximum 11-12 days after inoculation, and then declined (Fig. 3.5A). In the second experiment, mean sporulation was fairly constant over the first 20 days before rapidly decreasing (Fig. 3.5B). In both experiments, analysis of variance revealed that sporulation did not vary significantly with soil moisture but varied significantly over time ($P<0.001$). There was no significant interaction of treatment with time when data for all stress treatments were analyzed. At the first reading, spore number was significantly greater on leaf-rolled than unstressed plants ($P<0.05$) in the first experiment but not in the second experiment. The coefficient of variation was high in both experiments (31% and 56%).

Correlations between sporulation per unit sporulating area, lesion number, sporulating area, and senescence were similar for the two experiments. Spore number was negatively correlated with the total sporulating area of the leaf segment ($r=-0.19$ [$P<0.05$] and -0.22 [$P<0.05$] for the first and second experiment, respectively) but there was no correlation with lesion number or senescence. Sporulating area, lesion number and senescence were all positively correlated with each other ($r>0.23$ [$P<0.01$]) in both experiments.

3.4 Discussion

Relative infection efficiency was greater on expanding leaves than fully expanding leaves (Nottegham and Andriotempo 1979, Roumen 1993, Volk et al. 1958), and decreased on leaves at lower positions. Drought stress increased the leaf susceptibility period (Chapter 2), which was an important component in increasing total disease and the speed of disease progress (Chapter 3). Other components in the rice blast disease cycle also may be important for epidemic development. These include the rate and number of infections at different leaf wetness periods, lesion size, latent period and sporulation.

Because leaf wetness duration is highly variable in the field, the number of infections is dependent on the time required to complete the infection process. Very little research has been conducted to determine the effects of leaf postion and drought stress on the minimum leaf wetness duration required for infection. On plants at the eighth leaf stage, the minimum period of leaf wetness required was dependent on leaf position and drought stress (Fig 3.1). On plants at the fifth leaf stage, leaves at lower positions required longer leaf wetness durations than the expanding leaf (Figs 3.2 and 3.3). Pre-inoculation drought stress did not always have an effect on the minimum leaf wetness required for infection. However, when there was an effect, the time required for infection was reduced, with the effect tending to be greater for older than younger leaves. The differences between experiments may have been influenced by pre-inoculation environmental conditions which are an important cause of variability in rice blast research (Bonman et al. 1987).

It is possible that the differential effect of pre-inoculation drought stress on the rate parameter on older vs. younger leaves could be explained by the different requirements for hydrostatic pressure of appressoria attempting to penetrate different-aged leaves. The effects of drought stress on the rate parameter are likely mediated through the effects of drought on penetration and colonization (Kim 1985). There is some evidence which indicates that the differential effects of leaf position and drought stress on penetration is important. Indirect evidence based on differences in cuticular transpiration between fully-developed and expanding leaves (Yoshida and de los Reyes 1975) suggests that the barrier to infection is greater on older than younger leaves. A major component of this barrier to penetration is the leaf silica content (Volk et al. 1958). More direct evidence is available. The force required for mechanical penetration of the epidermis was greater for older than younger leaves (Suzuki 1965). In addition, more mechanical strength was required to penetrate unstressed than stressed plants (Suzuki 1965). Furthermore, with increased leaf wetness duration, the hydrostatic pressure in the appressoria increased (Howard et al. 1991). Thus, it may be hypothesized that, on younger leaves, regardless of whether they are stressed, appressoria, soon after formation, have adequate hydrostatic pressure to penetrate. In contrast, on older leaves, which inherently require appressoria to have considerably more hydrostatic pressure if they are to penetrate, the effect of drought stress becomes evident, with the proportion of infection sites being greater with drought stress.

The apparent increases in asymptotes with increasing leaf position and drought stress might indicate increases in the number of infection sites. It is possible that the number of infection sites is determined by the number of non-silicified cells. Leaf silica content and the number of silicified cells increased with leaf age. Infection frequency decreased with increasing leaf silica content (Osuna-Canizalez 1991, Volk et al. 1958, Ito and Sakamoto 1939, Suzuki 1963, Balasta et al. 1989). Furthermore, silica content and the number of silicified cells was less on plants grown on soil with low moisture than on plants grown in flooded soils (Ito and Sakamoto 1939, Suzuki 1963, Suzuki 1940, Kim 1985). In addition, drought stress could affect deposition of cell wall materials on expanding leaves. For example, in oat coleoptiles, cellulose fiber deposition decreased with decreasing water potentials (Cleland, 1967).

The effects of leaf position, cultivar and drought stress on infection efficiency, as a function of leaf wetness duration, need to be considered in simulation modelling. For example, predicted disease progress on a given leaf will vary depending on whether the infection rates are based on the results obtained from long wetness durations, or as a function of leaf wetness duration on individual leaves. In the first instance, the ratio of the infection efficiency on the most susceptible leaf to the infection efficiency on a less susceptible leaf would be similiar for all leaf wetness durations. However, this ratio would stabilize only at long leaf wetness durations such that there are no more infections with increasing leaf wetness duration. At leaf wetness durations shorter than that required to reach the asymptote, the ratio increases with leaf wetness duration because the rate parameter of the Richards model, an exponential function, varied with leaf position and drought stress (Tables 3.1, 3.2). Thus, it is likely that infection on individual leaves, or growth increments, as a function of leaf wetness duration, would better predict the disease severity on different plant parts.

Lesion size decreased slightly on older leaf parts (Figure 3.4) as reported earlier (Roumen 1993). However, intial lesion size was not affected by drought stress as previously reported (Gill and Bonman, 1988). There are two possible reasons why the results presented here differed from those of Gill and Bonman. First, a tendency for lesion size to increase with drought might have been compensated by competition among increasing number of lesions resulting from water stress. This seems somewhat unlikely given that there was a postive correlation between lesion density and lesions in this and other (Roumen 1993) experiments. Alternatively, the results could have occurred because of differences in experimental and sampling procedures. In my study, leaf-roll occurred within 12 days of the onset of drought whereas in Gill and Bonman's work, more time was required for leaf roll to begin. This difference could have altered the rate of some physiological processes, such as osmotic adjustment, to water loss.

In the *Magnaporthe grisea*-rice pathosystem, the incubation period and latent are period nearly coincidental (Roumen 1993, Yeh and Bonman 1985). Although there were significant differences in the latent periods over leaves of differing degrees of expansion, differences were small and were potentially confounded with lesion number since there are more lesions on young leaves. Latent period also varied with drought stress but once again, differences were small. Therefore, as has been reported earlier (Roumen 1993, Gill and Bonman 1988), changes in latent period resulting from drought stress or leaf age are likely to have little effect on the blast epidemiology on upland rice.

The methods for estimating sporulation were similar to those used by Kato and Kozaka (1975) in which different lesions were sampled over time. Their results indicated that sporulation reached a maximum after a few days and decreased rapidly, producing few spores thereafter. Using isolated lesions on a number of cultivars, including C22, Gill and Bonman (1989) reported similar results for unstressed plants and enhanced sporulation for plants subjected to pre-inoculation drought stress. Current models of rice blast (Calvero and Teng 1991, Gunther 1985) are based on measurements of sporulation of isolated lesions. The results presented here indicate that the values of epidemiological parameters derived from studies on isolated lesions may differ from those derived for groups of lesions. The numbers of spores per unit area and the total sporulating area per leaf area were negatively correlated, indicating possible competition or other interactions among lesions on the same leaf. For example, it is possible that a lesion on the main vein could greatly affect the sporulation of distal lesions.

In summary, pre-inoculation drought stress increased the number of lesions on expanding leaves, but the rate parameter of the Richards model was not greatly affected. Although the rate parameter was less on older than younger leaves, the reduction was greater on non-stressed plants. In contrast, there were no significant effects of pre-inoculation drought stress on initial lesion size, latency or spore production per unit area. Thus, the effect of a brief period of pre-inoculation drought stress on an epidemic is largely determined by the differential alteration of the relative infection efficiency on different leaves.

Table 3.1. Effect of drought stress (S0=saturation, S2=Leaf roll), leaf position and cultivar on the rate and asymptotes of the Richards model use to model transformed lesion density as a function of leaf wetness duration. A common shape parameter (1-m = 0.0155) was used.

Variety	Leaf	Soil moisture	Asymptote[#]	Rate	R^2
UPLRi5	5	S0	0.409[@]	0.611	0.84
		S1	0.614	0.611	
		S2	0.614	0.611	
	4	S0	0.091	0.353	0.81
		S1	0.552	0.635	
		S2	0.878	0.635	
C22	5	S0	0.768	0.639	0.94
		S1	0.917	0.713	
		S2	0.917	0.713	
	4	S0	0.303	0.364	0.82
		S1	0.548	0.661	
		S2	0.723	0.661	

[#] (Lesions/cm^2). Nonlinear regressions performed using treatment means.

[@] Unique estimates of the parameters reflect significant differences between stress levels ($P<0.05$)

Table 3.2. Effect of drought stress (S0=saturation, S4=Leaf roll) and leaf position on the rate and asymptotes of the Richards model use to model transformed lesion density as a function of leaf wetness duration. A common shape parameter (1-m)= 0.010 was used.

Leaf	Stress	Asymptote#	Rate	R^2
5	S0-S2	1.58@	0.524	0.94
	S3	2.73	0.524	
4	S0-S2	0.69	0.389	0.77
	S3	0.98	0.479	
3	S0-S2	0.14	0.365	0.71
	S3	0.14	0.365	

# (Lesions/cm^2). Nonlinear regressions performed using treatment means.

@ Unique estimates of the parameters reflect significant differences between stress levels ($P<0.05$)

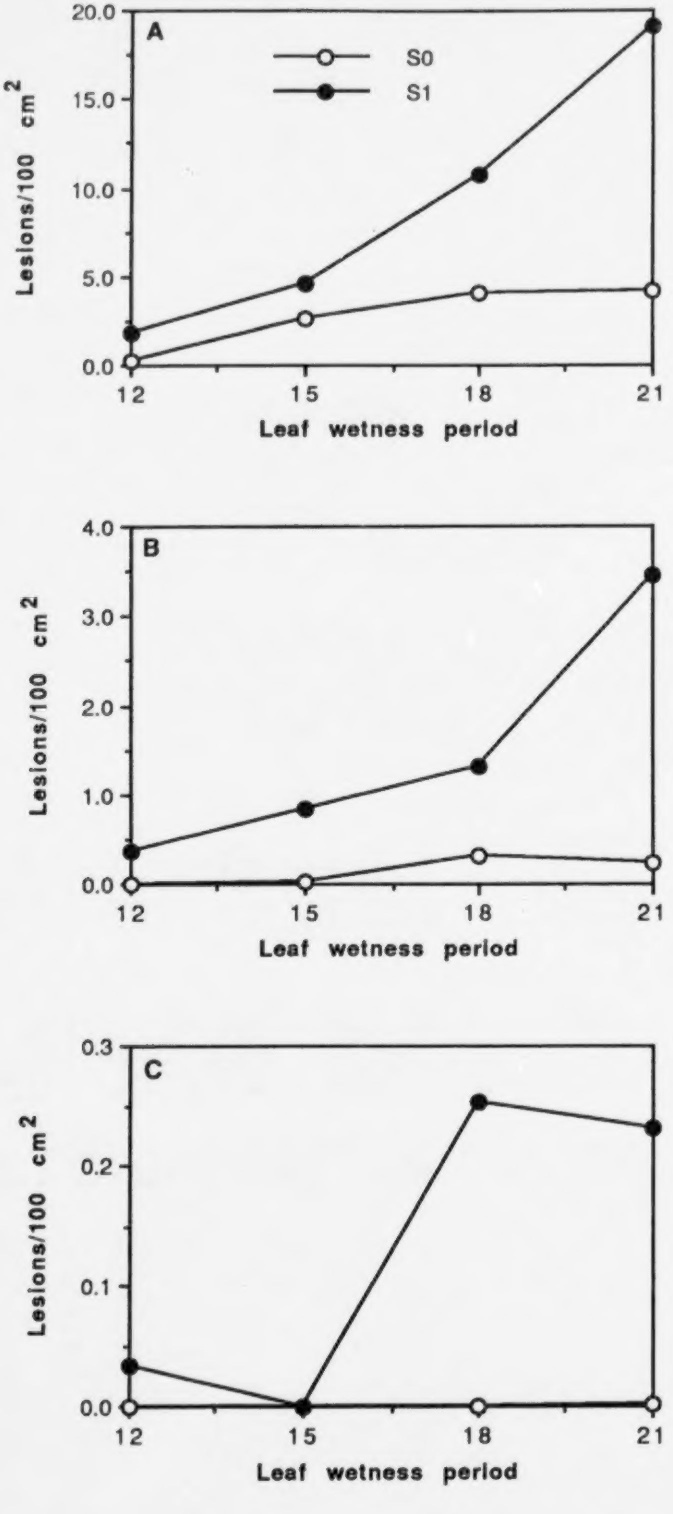
Table 3.3. Lesion number and latent period on different aged leaves.

		RIE@			Latent period		
Leaf age category#	N	Min	Max	Mean	Min	Max	Mean
0.00-0.30	74	0.1	57.9	10.5	84	120	96.4
0.30-0.60	61	0.4	4.6	1.9	84	128	110.0
0.60-1.00	133	0.2	5.5	1.3	84	128	108.5

@ Relative infection efficiency (lesions/10 cm^2)

# Proportion of the length to the final leaf length

Fig. 3.1. Effect of drought stress (S0=Saturation, S1=Leaf roll), leaf position and leaf wetness duration on relative infection efficiency (RIE) on plants at the eight leaf stage. A. Leaf 8. B. Leaf 7. C. Leaf 6. The F-values for interaction of stress with time (linear) for leaf positions 8 and 6 were both significant at the 0.05 level, respectively. The F-value for the main effects of stress and time on leaf position 7 was significant at the 0.001 and 0.05 levels, respectively. Note the difference in scales for RIE.


A
S0
S1
Lesions/100 cm^2
20.0
15.0
10.0
5.0
0.0
12
15
18
21
Leaf wetness period
B
Lesions/100 cm^2
4.0
3.0
2.0
1.0
0.0
12
15
18
21
Leaf wetness period
C
Lesions/100 cm^2
0.3
0.2
0.1
0.0
12
15
18
21
Leaf wetness period

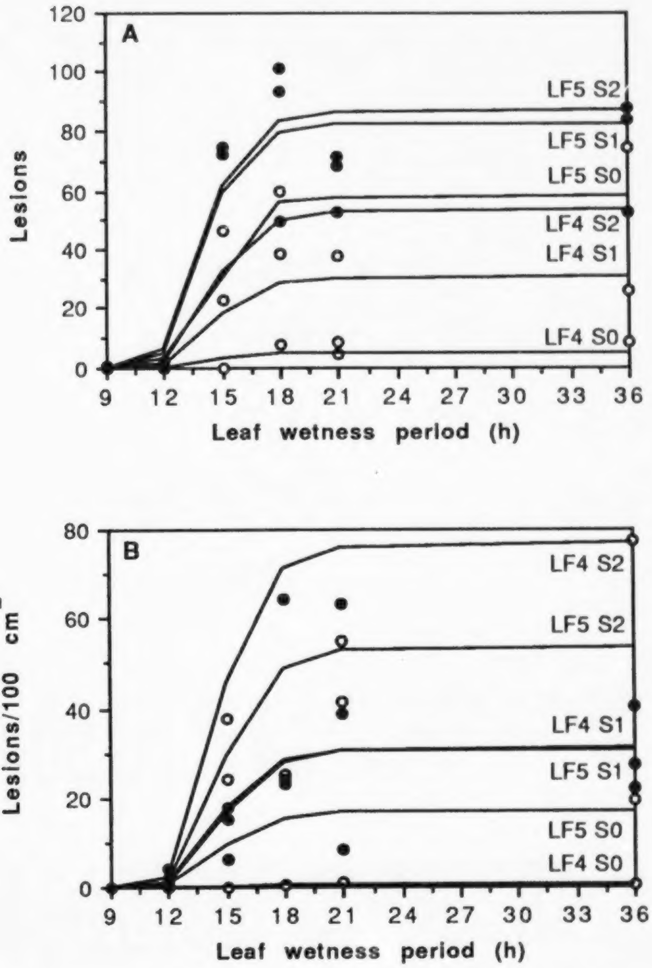
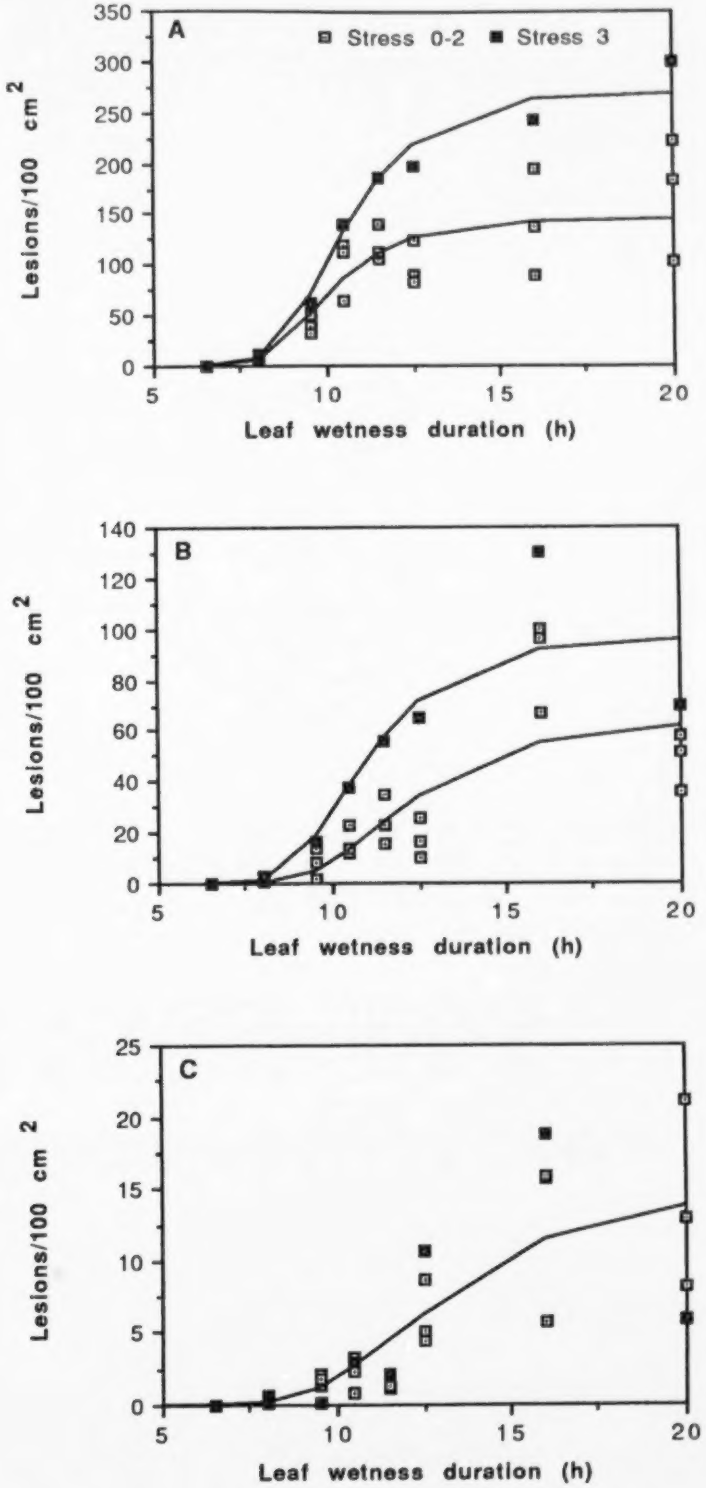


Fig. 3.2. Effect of drought stress (S0=saturation, S2=leaf roll), leaf position (LF4, LF 5), and leaf wetness duration on the number of susceptible lesions on C22 (A) and UPLRi5 (B). The F-values for the interactions of stress with time, cultivar with leaf position and leaf position with time were all significant at the 0.05 level. The lines are those predicted by nonlinear regression.

Fig. 3.1. Effect of drought stress (S0=Saturation, S1=Leaf roll), leaf position and leaf wetness duration on relative infection efficiency (RIE) on plants at the eight leaf stage. A. Leaf 8. B. Leaf 7. C. Leaf 6. The F-values for interaction of stress with time (linear) for leaf positions 8 and 6 were both significant at the 0.05 level, respectively. The F-value for the main effects of stress and time on leaf position 7 was significant at the 0.001 and 0.05 levels, respectively. Note the difference in scales for RIE.


A
Stress 0-2
Stress 3
Lesions/100 cm^2
350
300
250
200
150
100
50
0
5
10
15
20
Leaf wetness duration (h)
B
Lesions/100 cm^2
140
120
100
80
60
40
20
0
5
10
15
20
Leaf wetness duration (h)
C
Lesions/100 cm^2
25
20
15
10
5
0
5
10
15
20
Leaf wetness duration (h)

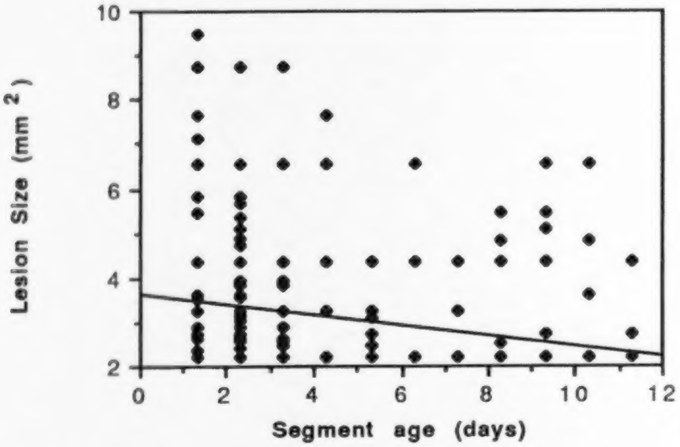


Fig. 3.4. Effect of leaf segment on initial lesion size. The leaf segment effect, but neither the stress effect nor the stress with leaf segment interaction, was significant at the 0.05 level.

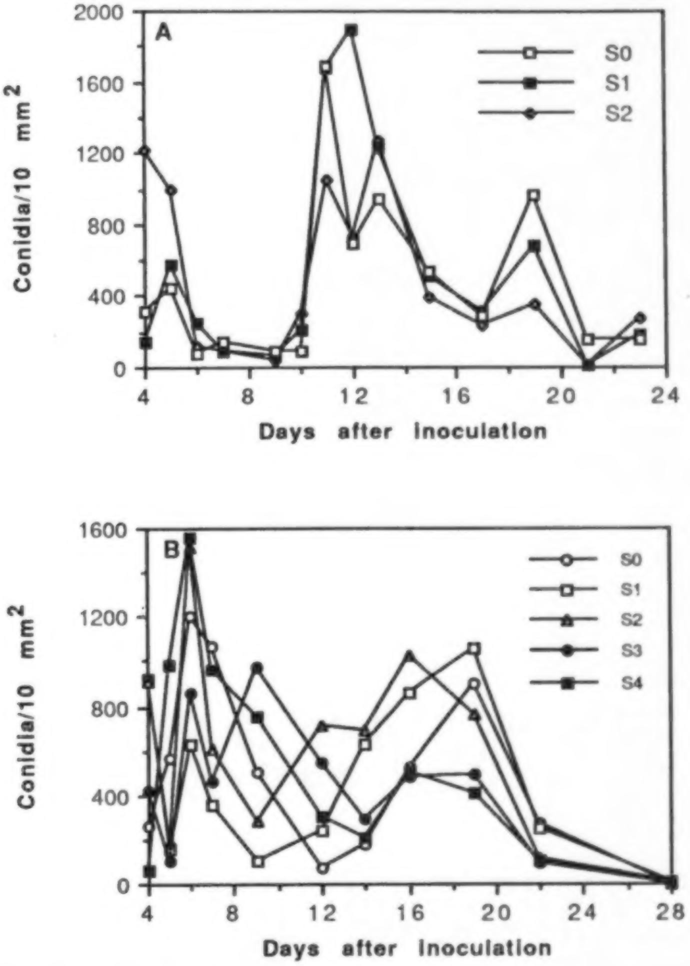


Fig 3.5. Effect of drought stress on sporulation over time. A. Experiment 1 (S0=saturation, S2=Leaf roll). B. Experiment 2 (S0=saturation, S4=Leaf roll). In both experiments, the F-value for the time effect was significant (P=0.001).

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Chapter 4. Effect of infection efficiency decreasing with tissue age on disease progress: A simulation study.

4.1 Introduction

Modelling is an intrinsic part of systems analysis, a methodology that facilitates logical and coherent decision making (Teng 1985, Seem and Haith 1986). Models, analytic or simulation, have been used in the development of theory (Jeger 1984, Jeger and van den Bosch 1994), to examine the relative importance of different epidemiological parameters (Zadoks 1971), as the basis for determining which parameters should be used for comparing epidemics (Madden 1986), and in disease management (Jeger 1986, Zadoks 1990).

Although generalized growth models, such as the logistic, Gompertz and Richards models, have been used for describing disease progress curves, these models may be somewhat unrealistic since epidemiological and plant growth parameters are not included (Berger 1989). Some of these deficiencies were overcome in a general model for disease progress on growing plants (Berger and Jones 1985). Although more realistic, this model described disease progress on the whole plant or crop canopy. In other models, the leaf canopy was broken into distinct layers to measure the effects of disease on the crop (Waggoner 1990). For modelling disease progress, it may be more useful to include another level of detail, disease progress on the daily increments of growth (Berger 1989).

A crop cultivar has partial resistance if it is susceptible to infection, yet has a reduced rate of epidemic progression (Parlevliet 1979). The assessment of partial resistance is made by measuring the components of the infection cycle that reduce overall disease severity. These components include infection efficiency, the proportion of spores that produce sporulating lesions; latent period, the time from infection to sporulation; spore production, the number of spores produced per unit area of infected tissue; and infectious period, the period during which a lesion sporulates. All of these mechanisms of partial resistance are available to the plant breeder. Because disease severity at any one time is not a simple function of these components, simulation can be used to determine which are the most effective.

Infection efficiency often varies with tissue age, with biotrophic fungi generally more likely to infect younger tissue and perthotrophic fungi generally more likely to infect older tissue (Zadoks and Schein 1979). In the *Oryza sativa* L. - *Magnaporthe grisea* (Herbert) Barr pathosystem, infection efficiency was greater on younger plants and on younger tissues at a given plant age (Kahn and Libby 1959, Roumen 1993). Furthermore, these age-effects varied with cultivar and were important components of partial resistance (Roumen 1993, Koh et al. 1987).

There were three objectives in the project reported here. The first was to develop an improved model of disease progression which incorporated changes in duration of susceptibility to infection, based on the cohort model of Berger (1989). The second was to compare the relative effectiveness of reduced duration of susceptibility and other resistance mechanisms to determine which would likely be the most effective in disease control. The final objective was to examine the interactions between the different host and pathogen parameters to gain a better understanding of rice blast progression.

4.2 Model Description

Host Submodel. The model assumed a single canopy layer. The host grew according to the model described by Berger and Jones (1985) with a modification to facilitate the simulation of disease on tissues of different ages. The model included a vector DILA(j) representing the increase in leaf area at time i = j (Fig. 4.1). Leaf growth at time i was calculated as the product of an intrinsic growth rate, the proportion of maximum leaf area not yet attained, the proportion of the simulation duration not yet attained and the amount of healthy leaf area. The second proportion was included to reduce the compensation effect of small total leaf areas in simulations with large amounts of disease. The healthy leaf area was calculated as the sum of the differences between the total and diseased areas in the i-1 elements of DILA(·) . In notation,

$$[4.1]\quad LA(i)=LA(i-1) + K_H \cdot \left(1-\frac{LA(i-1)}{LM}\right)\cdot\left(1-\frac{i}{i_{MAX}}\right)\cdot \sum_{J=1}^{I-1} DILA(J)-YNT(J)$$

where $LA(i)$ is the total leaf area at time i, K_H is the intrinsic growth rate, LM is the maximum leaf area, i_{MAX} is the total time period over which crop growth is to be simulated, DILA(j) and YNT(j) are the total and diseased leaf areas, respectively, of the jth element of DILA(·)

Pathogen submodel. Onset of the epidemic was simulated by an initial amount of inoculum arriving from an outside source. Spores were assumed to disperse uniformily over the entire simulated field which had an area equal to the maximum leaf area, LM. Thus, an equal number of spores per unit area was deposited on each element of DILA(·)

Infection, lesion growth and sporulation were calculated separately for each of the i elements of DILA(·) and summed over all i elements. At the time of infection, all new lesions had a small initial lesion size, Z_0. At time i, the area and number of new infections on the jth element of DILA(·) were

[4.3] $$\mathrm{LESAREA}(1,j) = Z_0 \cdot \mathrm{IE} \cdot \mathrm{SPOR}(i-1) \cdot \frac{\mathrm{DILA}(j)}{\mathrm{LM}} \cdot \left(1 - \frac{\mathrm{YNT}(j)}{\mathrm{DILA}(j)}\right) \text{ and}$$

[4.4] $$\mathrm{LESNO}(1,j) = \frac{\mathrm{LESAREA}(1, j)}{Z_0},$$

respectively, where IE is the infection efficiency, and SPOR(i-1) is the number of spores available for infection at time i and DILA(·)/LM simulates uniform spore distribution. The number of new lesions on segment j [LESNO(1,j)] was calculated indirectly by dividing the total area at infection by the size of those lesions (Z_0).

Lesion growth was modelled using the logistic equation with common rate and maximum lesion size parameters for each leaf segment, but separate values for maximum susceptible tissue and diseased tissue, reflecting differences in total and diseased leaf area for each element of DILA(·). Lesion area for lesions of age k-1, k = 2, i-T_0, (where T_0 is the crop age at the time of epidemic onset) was calculated as:

[4.5] $$\mathrm{LESAREA}(k,j)=\mathrm{LESAREA}(k-1,j)+K_Z \cdot \mathrm{LESAREA}(k-1,j) \cdot \left(1 - \frac{\mathrm{YNT}(j)}{\mathrm{DILA}(J)}\right) \cdot \left(1 - \frac{\mathrm{LESAREA}(k-1,j)}{\mathrm{LESNO}(k-1, j) \cdot Z_{MAX}}\right),$$

where LESAREA(k, j) and LESNO(k,j) are the lesion area and lesion number of age (k-1) on the jth element of DILA(·) at time i (i=1 to i_{MAX}) and k=(2, i). K_Z and Z_{MAX} were parameters for lesion growth rate and maximum lesion size, respectively. At the same time i, LESNO(k,j) lesions of age (k-1) were advanced to LESNO(k,j). Then total diseased area for each element of DILA(·) was the sum of the area of all lesions:

[4.6] $$\mathrm{YNT}(j) = \sum_{k=1}^{i} \mathrm{LESAREA}(k, j).$$

Finally, spore production was proportional to lesion size, but declined linearly from a maximum at the end of the latent period to zero at the end of the infectious period. The equations for sporulation were:

$$[4.7a] \qquad TOT(j) = \sum_{k=LP}^{LP+IP-1} LESAREA(k,j) \cdot S_Z \cdot \left(1 - \frac{k-LP}{IP}\right)$$

$$[4.7b] \qquad SPOR(i) = \sum_{j=1}^{i} TOT(j)$$

where TOT(j) is the total number of spores on the jth element of DILA(·), SPOR(i) is the total number of spores formed at time i, S_Z is the number of spores per unit lesion area, LP is the latent period, and IP is the infectious period.

During each time unit, after lesion growth and new infections were calculated on each of the i elements of DILA(·), total diseased area at time i, Y(i), was calculated by summing over all i elements of YNT(·). Then, disease severity for the simulated field was calculated by dividing Y(i) by LA(i).

Modelling changes in susceptibility with tissue age. The above model for plant growth allowed for the differences in susceptibility between segments to be modelled. This was done by multiplying the right hand side of [4.3] by a factor S(·), which reduced the infection efficiency as a consequence of tissue ageing. Any given segment of DILA(·) was assumed to remain susceptible for a period of time SUSPER. S(·) decreased linearly from a value of 1 for a newly formed segment to a value of 0 for a segment of age SUSPER. Specifically,

$$[4.8] \qquad S(j) = \begin{cases} 1 - \dfrac{j}{SUSPER} & \text{for } j = 0.\ SUSPER \\ 0 & \text{otherwise} \end{cases}$$

It should be noted that whereas infection efficiency was modified by changes in susceptibility, inherent rates of lesion growth were constant at all times and sporulation per unit area was modified by lesion age only.

Modelling changes in susceptibility with plant age. Changes in susceptibility due to plant age, i, were incorporated into the model by multiplying the right hand side of [4.3] by a factor PLAR(i). PLAR(·) declined linearly from unity at epidemic onset T_0 to zero at the end of the simulation. Mathematically,

$$[4.9] \quad \text{PLAR} = 1 - \frac{(i - T_0)}{(i_{MAX} - T_0)} .$$

4.3 Results

Impact of susceptibility changes due to tissue age. Although simulation modelling provides a method for evaluating the effects of tissue-age-related susceptibility, explicit solutions are possible only if simplifying assumptions are made. For example, the importance of tissue age related susceptibility may be studied when YNT(·) << DILA(·), such that pathogen growth is not limited by availability of susceptible tissue and DILA(i) is a constant, LD, for all i. To simplify, I used the following equation with the apparent infection rate, KD, to increase potential diseased area and a maximum field size for distributing the potential diseased area. Then:

$$[4.10] \qquad Y(i) = Y(n-1) + \sum_{j=0}^{SUSPER} S(j) \cdot KD \cdot Y(i-1) \cdot \frac{DILA(i+j)}{L_{MAX}}$$

where i > SUSPER.

It can be shown, using the above definition for S(j), that:

$$[4.11] \qquad Y(n) = Y(n-1) \cdot \left(1 + KD \cdot \frac{SUSPER+1}{2} \cdot \frac{LD}{L_{MAX}}\right)$$

$$= Y(n-1) \cdot (1 + (KD \cdot (SUSPER+1) \cdot m)$$

where $m = \dfrac{LD}{2 \cdot L_{MAX}}$.

Furthermore, if S(j) = 1 for all j=1, n, that is, all tissue remains equally susceptible, then

$$[4.12] \qquad Y(i) = Y(i-1) \cdot (1 + KD \cdot i \cdot m)$$

In situations where an epidemic is in the logarithmic phase, and KD is adjusted by decreasing tissue susceptibility, the rate of increase (1+r) is constant from one generation to the next whereas in an epidemic where KD is not so conditioned, the rate is increasing and is a function of the increase in leaf area. Under both assumptions, increasing the size of DILA(·) relative to the maximum leaf area increases the rate. However, if leaf area does not increase linearly, then the infection rate depends on tissue growth in both cases.

Susceptibility related to tissue age. Infection efficiency was assumed to decrease linearly from a maximum IE at leaf segment emergence to zero at time SUSPER after leaf segment emergence. Increasing the susceptibility period, SUSPER, increased final disease levels, and decreased the time to the inflection point (which occurred at higher disease severities with increasing SUSPER)(Fig. 4.2A). At low values of SUSPER, the trajectory of the epidemic initially was parabolic with disease increasing for 4 days and decreasing thereafter (Fig. 4.2B).

Disease progress on individual growth increments is illustrated in Fig. 4.3. In simulations of $S(j) = 1$ (ie, all segments were fully susceptible), disease progressed most rapidly on increments formed on days 60-65, somewhat less rapidly on increments formed afterwards, and considerably more rapidly on increments formed beforehand. In this situation, disease progress was constrained only by the availability of host tissue. Including SUSPER = 20 and S(j) in the model reduced the rate of disease on most segments with the effect being greatest on the earlier formed segments. Further reductions in SUSPER slowed the epidemics on individual growth increments.

Increasing the susceptibility period from 4 to 20 days increased the initial disease severity by increasing the proportion of susceptible tissue at the onset of the epidemic (Fig. 4.4). The amount of this increase was, however, dependent on how host growth was modelled. If, instead of growing logistically prior to the beginning of the epidemic, host tissue increased by a constant amount in each time period, then the initial disease severity was proportional to (SUSPER + 1)/2. Furthermore, as long as the time of epidemic onset was greater than SUSPER, the initial disease severity did not vary with the time at which the epidemic began because the amount of susceptible tissue was constant. In contrast, if the host had undergone logistic growth prior to the onset of the epidemic, the initial disease severity varied with the time of infection because the size of the susceptible segments also varied with time.

To test the relative importance of IE and SUSPER, these two parameters were varied such that the product IE · (SUSPER + 1) was a constant. In the first series of simulations, the overall initial disease severity was constant and distributed among the susceptible segments according to their size and SUSPER. Increasing SUSPER and decreasing infection efficiency decreased disease severity for the first 40 days of the epidemic (Fig. 4.5A). The slopes of the disease progress curves at 60 days and the final disease severity increased as SUSPER increased. Because IE · (SUSPER+1) was kept constant, the infection efficiency decreased with increasing SUSPER; nevertheless, higher values of SUSPER were associated with more susceptible tissue. Thus, the number of new infections at the end of the epidemic increased with increasing SUSPER. Although increasing SUSPER significantly increased the amount of diseased tissue at the end of the epidemic, it had much less effect on the amount of healthy area of the simulated crop; the area under the healthy area curve for SUSPER = 20 was approximately 101% of the area for SUSPER = 4.

In a second series of simulations, the initial inoculum load was constant and the initial diseased area was permitted to vary with susceptibility duration. Disease progress curves were approximately coincidental until 42 days after epidemic onset (Fig 4.5B). At this point, the disease progress curves diverged and final disease severity was greater for longer susceptibility periods and lower infection efficiencies (Fig. 4.5B). In contrast to the previous series, area under the healthy area curve decreased with increasing SUSPER. The proportion of the area under the healthy area curve of the simulated crop for SUSPER = 20 was 86% of the area for SUSPER = 4.

The effect of changes in the period of leaf susceptibility and host growth rate was examined under conditions where host growth was increased such that the maximum leaf area was reached well before the end of each simulation. In simulations where all segments were fully susceptible [S(j)=1], disease severity increased with increasing host growth rate (Fig. 4.6). In simulations where leaf age resistance (SUSPER) was included, initial disease progress rates increased with increasing host growth rates, but final disease severity decreased with increasing host growth rates.

To determine the importance of susceptibility period; relative to infection efficiency, lesion growth rate, maximum lesion size, latent period and infectious period; in determining disease progress, the parameters were varied, one at a time, by 20%. Because the number of new infections is proportional to spore production density, initial lesion size, and infection efficiency (equation 4.3), the effects of these three parameters are equivalent. Thus, for the sake of simplicity, only an increase in infection efficiency was simulated.

Increasing the susceptibility period increased the rate of disease progression and final disease severity (Table 4.2). Only infection efficiency, latent period and maximum lesion size had similar effects on the simulated epidemic; differences in the effects of these parameters were small (Fig. 4.7). Although the effect of increasing SUSPER was small relative to infection efficiency, latent period and maximum lesion size in the early phases of the simulated epidemic, final disease severities were similar for all four parameters. The effect of decreased latent period was dependent on the lesion growth rates. At low lesion growth rates, decreasing the latent period had little effect; although sporulation was initiated earlier, spore production was reduced due to a smaller lesion size at the end of the latent period. In contrast, at relatively high lesion growth rates, such that lesion sizes at the end of different latent periods were similar, a decrease in the latent period increased the epidemic rate dramatically.

Because the effect of latent period was so dependent on the rate of lesion expansion, further simulations were done in which lesion size was held constant by setting the initial lesion size equal to its maximum lesion size. The greatest increase in final disease severity occurred in response to an increase in susceptibility period although decreasing the latent period, and increasing the infection efficiency also increased the amount of disease.

Plant age resistance. Including plant age resistance decreased disease severity at all times for all values of SUSPER (Fig 4.8). In addition, the relative importance of plant age resistance measured by the ratio of simulated final disease severity with plant age resistance to simulated final disease severity without plant age resistance, decreased as SUSPER decreased.

Interruption in infection. A brief period of unfavorable conditions for infection was simulated by setting the infection efficiency to zero for a period of 5 days beginning 8 days after onset of the epidemic (Fig. 4.9). The decrease in disease severity was initially slow because lesion growth was permitted, although there were no new infections. Two days after the interruption began, disease severity decreased more rapidly because host growth was relatively greater than lesion growth. After the 5 day period of unfavorable conditions for infection, disease severity continued to decrease for another 2 days because the host growth was relatively greater than the growth of new infections and the latent period required for these new infections to begin sporulation (Fig. 4.9A). Disease severity was always less for interrupted than uninterrupted epidemics. The relative disease severity, which is the ratio of the interrupted and uninterrupted epidemics, decreased more rapidly for SUSPER = 20 than SUSPER = 10 following onset of the interruption (Fig 4.9B), but increased more rapidly for SUSPER = 20 at the end of the epidemic. These trends were evident for all times of interruption onset.

Interaction between components of resistance. In a series of simulations, the interactions of the parameters SUSPER, IE and Z_{MAX} were examined, in a three factor factorial design with two levels per factor. Plant age resistance was included as a fourth factor by repeating the simulation with the linear function for PLAR. The area under the disease progress curve was calculated by rectangular integration. The AUDPC's were plotted against the number of parameters that were increased simultaneously (Fig. 4.10). Increasing SUSPER, IE, and Z_{MAX} individually 20% increased AUDPC. The AUDPC increased further by increasing two of the three parameters simultaneously. The largest AUDPC was obtained when all parameters were increased by 20%. These effects were approximately additive.

The AUDPC was reduced by including plant age resistance, but the effect of PLAR depended on SUSPER. For SUSPER = 20 increasing the parameters led to similar increases in AUDPC for simulations with and without PLAR (Fig 4.10). In contrast, for SUSPER = 10, increasing the parameters produced smaller increases in AUPDC for simulations with PLAR than simulations without PLAR.

4.4 Discussion

The model used by Berger and Jones (1985) to describe disease progress on growing hosts was more complex than the simple and versatile model used by Vanderplank (1963). This model was more realistic biologically, and significantly improved the interpretation of disease progress curves. However, their model described disease progress at the total population level of the host. Berger (1989) improved the model further by monitoring disease on each cohort of leaf area. By including disease progress on individual increments of growth, the model can be used to study disease progress in pathosystems, such as rice blast, in which host susceptibility changes greatly over time.

The current model could be used as a basis for predicting disease progress on different segments. Techniques exist for predicting disease severity on individual leaves from whole plant data (Subbarao et al. 1989). In that model, the disease progress curves on individual leaves were assumed to have similar disease progress curves. However, our simulations showed that disease progress varied with growth increment (Fig. 4.3). This occurred because the inoculum load varied with disease progress of the simulated crop. Thus, modelling disease progression on the leaf cohorts provides an alternative for estimation of disase severity on different leaf layers for simulation of yield loss.

Simulated epidemics were delayed because all inoculum was dispersed over the entire simulated area which was equivalent to the carrying capacity of the host. As the host area increased, a larger proportion of dispersal units landed on host tissue. A more realistic model would include factors that affect deposition in a multi-layered canopy, such as the leaf angle, the height of the emerging leaf relative to the position of the height of the infectious lesions and the distribution of the inoculum within the canopy (Gunther 1986, Koizumi and Kato 1991).

There is some experimental evidence that duration of susceptibility is important in the epidemiology of rice blast. For example, rice cultivars were found to differ in the rate at which individual leaves became resistant to infection (Roumen et al. 1992). Secondly, the number of leaves on the main culm with sporulating lesions was correlated with area under the disease progress curve for the six cultivars used in field trials in Bonman et al. (1989). Finally, relative infection efficiency, as affected by leaf age, was an important component of partial resistance (Roumen 1993). Through simulation modelling, I have extended these results to include multiple infection cycles and demonstrated that decreasing the length of the susceptibility period to infection reduced the rate of the epidemic. Decreasing the susceptibility period S by n time units decreased the rate during the exponential phase by a factor of (S-n+1)/(S+1). If S is small, changes in the susceptibility period dramatically reduce the rate of disease progress. For example, changing the susceptibility duration from 10 to 9 reduces the rate by 10%, whereas a similar reduction from 20 to 19 reduces the rate by only 5%.

Not surprisingly, including plant age resistance in the simulations decreased disease severity further. In the simulations, however, there was an apparent interaction between plant age resistance and tissue age resistance, particularly on tissue which became resistant within a short period after tissue emergence (Fig. 4.10). This interaction was apparent only at smaller values of SUSPER. Thus, increasing leaf age related resistance is likely to improve models coupling host and pathogen dynamics.

Previous simulations which used different models, including the logistic equation, to model both host and parasite growth, indicated that increasing growth rates of either component increased disease severity over the entire epidemic (Berger and Jones 1985). The current model produced similar results if all tissue was equally susceptible. However, if tissue susceptibility decreased with time, increasing the host growth rate increased disease severity initially, but reduced final disease severity (Fig. 4.6). This initial increase in disease resulted from an increase in the number of lesions per crop area at the onset of the epidemic, due to a larger host leaf area. Disease severity continued to increase rapidly because total susceptible tissue was greater in simulations with higher than lower host growth rates. Later, the development of new host area slowed as the carrying capacity was approached. Thus, in simulations with greater host growth rates, greater number of spores were deposited on relatively or completely resistant tissue in the later phases of the epidemic.

In simulations with a constant lesion size, epidemic development was most affected by infection efficiency and maximum lesion size and about equally affected by change in segment susceptibility period and change in latent period. In contrast, in simulations including lesion growth rates, a change in susceptibility period was more sensitive than a change in latent period.

When selecting for partial resistance, it is possible that any gains made by selecting for shorter susceptibility periods may be offset by increases in infection efficiencies or changes in other epidemiological parameters. However, in monocyclic inoculation trials, lower infection efficiencies on the expanding leaves of ten rice cultivars were correlated with shorter susceptibility durations (Roumen 1993). Furthermore, in simulations in which the products of susceptibility period and infection efficiency were constant, final disease severity decreased with decreasing susceptibility duration (Fig. 4.5).

After a period of interrupted infection, subsequent disease development was greater than prior to the interruption (Berger and Jones 1985). In their simulations, disease levels in interrupted epidemics approached or surpassed the disease levels of uninterrupted epidemics. However, in my simulations, the increase in disease after the interruption, which occurred because of an increase in the healthy leaf area available for infection, was offset by an increase in the rate at which the tissue became resistant (Fig. 4.9), Perhaps most importantly, although infection interruption shifted disease progress to the right, the effect was relatively consistent across different levels of susceptibility duration. Thus, although the effects of susceptibility duration were examined primarily under the assumption of constant IE, these effects would likely be realized under conditions of fluctuating IE as well.

Although a decrease in latent period increased disease severity in the early phases of an epidemic, final disease severity increased only slightly and occasionally even decreased in a few simulations, depending on the values of the other parameters. This occurred because, with shorter latent periods, lesions were younger and therefore smaller during their sporulation period. This phenomenon could be avoided by assuming a constant lesion size. Alternatively, correction factors could be used in the form of increased sporulation capacity or lesion growth rates to overcome the negative effects of shorter latent periods on lesion size and consequently sporulation. However, the lesion growth rate would have to increase to an order of $(1+K_Z)^{m/n}$, if lesion growth is exponential, to achieve a lesion size such that sporulation per area of lesion for lesions at the end of latent period n would be similar for lesions with a longer latent period m. For example, given a lesion growth rate of 3 units/ day and a latent period of 4 d, the K_Z

would have to increase from 3 to 15 to compensate for a reduction in latent period from 4 d to 2 d. It is questionable whether such a large increase in lesion growth rates is biologically realistic.

In this paper, I refined the disease progress model of Berger (1989) to include the susceptibility duration of plant parts and determined the effect of this parameter on diease progression. The simulations demonstrated that the susceptibility duration was as important as infection efficiency, latent period and the maximum lesion size in determining disease progress. Thus, breeders can have confidence, that in pathosystems where susceptibility duration is variable, by selecting lines with reduced susceptibility duration they will be able to reduce epidemic development.

Table 4.1. List of parameters with inital settings and dimensions used in the simulation model.

Parameter	Description	Value	Dimensions[†]
i_{MAX}	Simulation time	80	T
K_L	Host growth rate	0.225	T^{-1}
L_{MAX}	Host carrying capacity	10,000	L^2
L_0	Host area at t = 0	10	L^2
T_0	Epidemic onset time	21	T
Y_0	Diseased area at t=21	0.0001	L^2
IE	Initial infection efficiency	0.04	1
LP	Latent period	5	T
Z_0	Initial lesion size	0.0001	L^2
Z_{MAX}	Maximum lesion size	0.01	L^2
K_Z	Lesion growth rate	4	T^{-1}
IP	Infectious period	20	T
S_Z	Spores/ unit lesion area	10,000	$N \cdot L^{-2}$
SUSPER	Susceptibility duration	20	T
PLAR	Susceptibility due to plant age	1.	1

[†] T = time, L = length, N = number, 1 = dimensionless

Table 4.2. Effect of changing the initial parameters by 20% on final disease severity and area under the disease progress curve (AUDPC [days]). The initial values for SUSPER and LP were 15 and 5 days, respectively. The initial settings for other parameters are listed in Table 1. Initial inoculum was distributed equally over all segments. All parameters were increased by 20% except LP which was reduced by 20%.

Parameter	Severity	AUDPC
Initial	0.40	7.69
SUSPER	0.51	10.54
IE	0.48	10.39
LP	0.48	10.21
IP	0.42	8.48
K_Z	0.40	7.76
Z_{MAX}	0.51	11.22

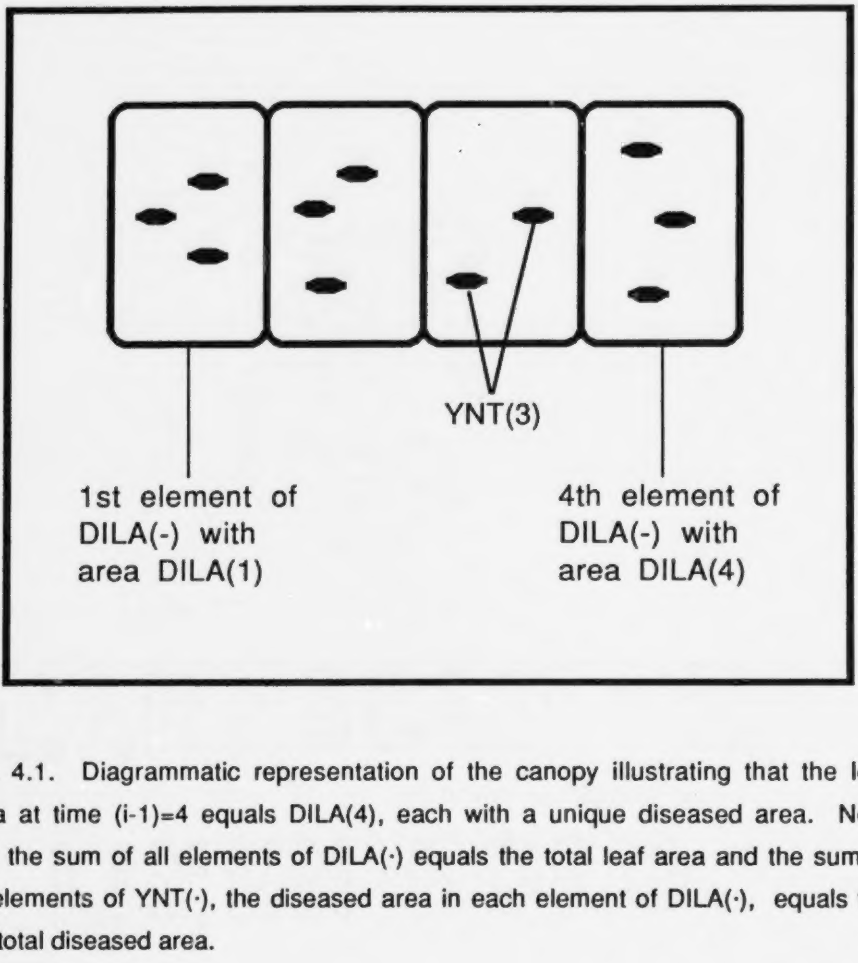
YNT(3)
1st element of DILA(-) with area DILA(1)
4th element of DILA(-) with area DILA(4)

Fig. 4.1. Diagrammatic representation of the canopy illustrating that the leaf area at time (i-1)=4 equals DILA(4), each with a unique diseased area. Note that the sum of all elements of DILA(·) equals the total leaf area and the sum of all elements of YNT(·), the diseased area in each element of DILA(·), equals the the total diseased area.

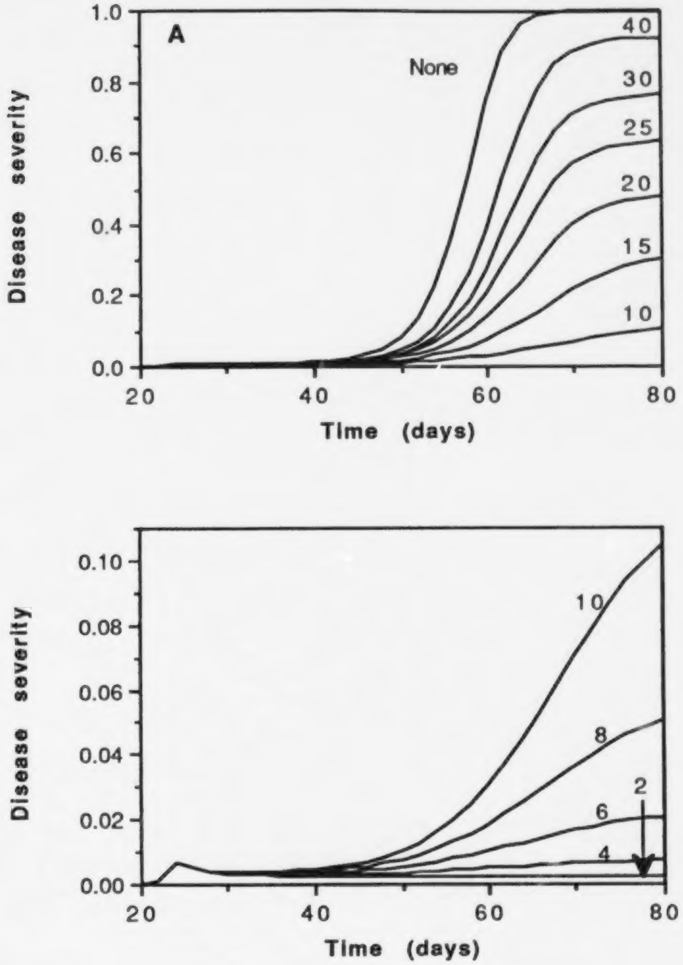

Fig. 4.2. Effect of susceptibility durations (SUSPER) of 5 - 40 (A) and 2 - 10 (B) on simulated disease progress. "None" indicates no change in susceptibility such that S(j) = 1 for all segments at all times. Susceptibility decreased linearly from the maximum infection efficiency (IE) at time of appearance of the growth increment to zero at SUSPER days later. Settings of other parameters are listed in Table 4.1.

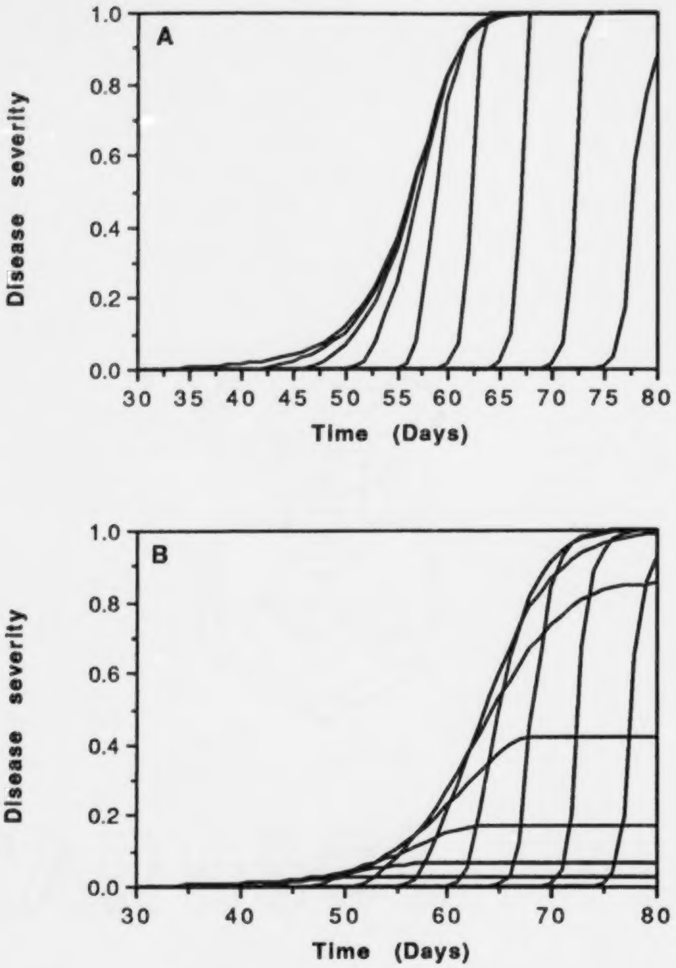

Fig. 4.3. Simulated disease progress on nine different growth increments formed at times 30-75 days. The growth increments were either equally susceptible for the duration of the simulation (A) or underwent a linear decrease in susceptibility with SUSPER = 20. Settings of other parameters are listed in Table 4.1.

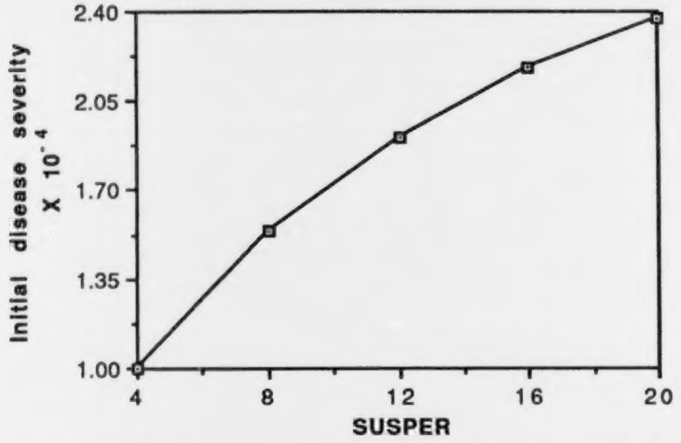

Fig. 4.4. Effect of susceptibility duration (4-20 days) on initial disease severity with initial inoculum arising from a spore cloud. The spore cloud at the onset of the epidemic gave rise to an initial disease severity of 0.0001 for SUSPER = 4. The initial disease severity on the youngest segment was held constant for all simulations. Settings of other parameters are listed in Table 4.1.

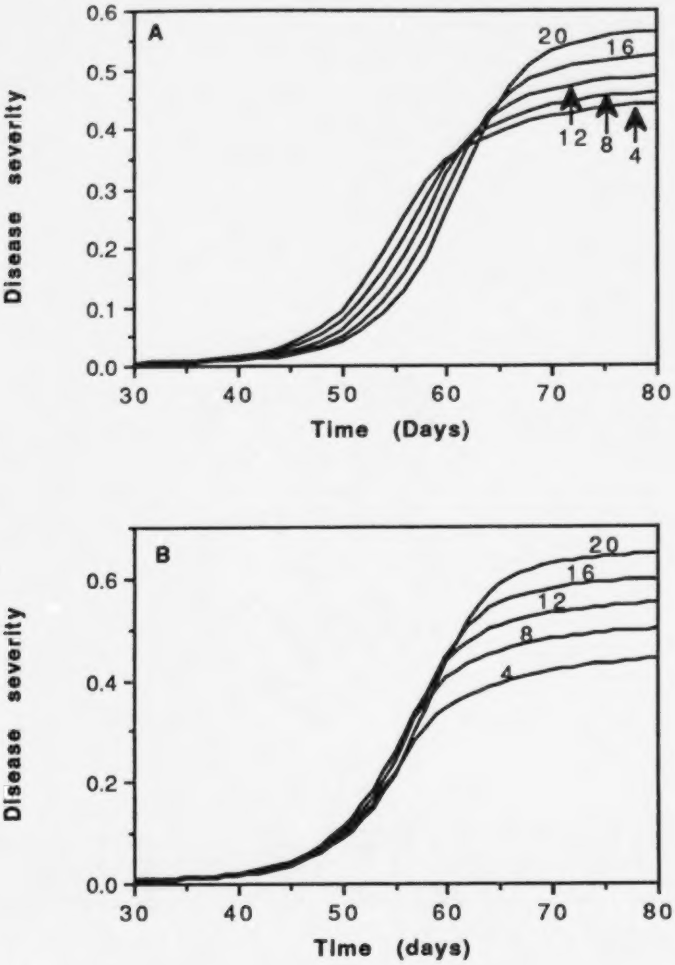

Fig. 4.5. Effect of varying infection efficiency (IE) and susceptibility duration (SUSPER=4 to 20 days) on simulated disease progress. (A) The overall initial disease severity was constant at 0.0001 and distributed among the segments according to their size and SUSPER. (B) The initial inoculum load was constant and the initial disease severity was permitted to vary with susceptibility duration. Simulated SUSPER values are illustrated in the graph. The multiple (SUSPER + 1)*IE was constant at 0.84. Settings of other parameters are listed in Table 4.1.

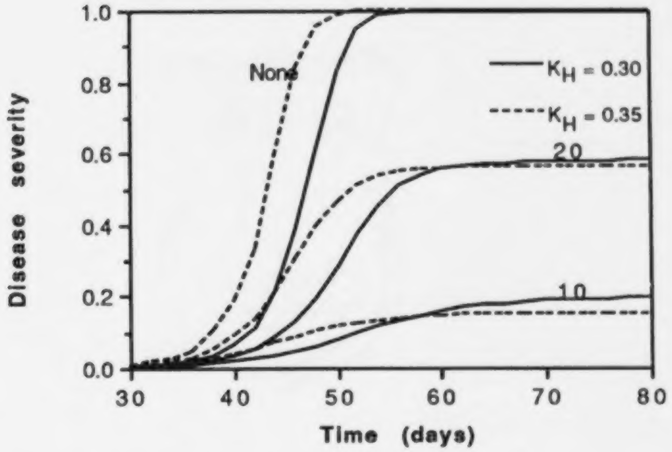

Fig. 4.6. Effect of two host growth rates (K_H = 0.30 or 0.35) and three susceptibility periods (SUSPER = 10 d, 20 d or None) on simulated disease progress on the crop. Each simulation run used an initial disease severity of 0.0001. "None" indicates no change in susceptibility such that S(j) = 1 for all segments at all times. Settings of other parameters are listed in Table 4.1.

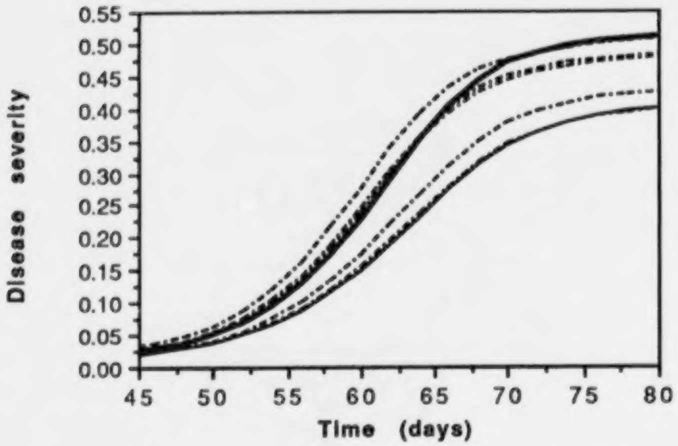

Fig. 4.7. Effect of 20% changes in susceptibility period (SUSPER) [——] and infection efficiency (IE), latent period (LP), infectious period (IP), lesion growth rate (K_Z) and maximum lesion size (Z_{MAX}) [----] showing that the relative effect of SUSPER was greater at the end than beginning of the epidemic. The simulated disease progress for the initial parameter settings is illustrated by the lowermost curve. All parameters were changed by 20%. Initial SUSPER AND LP were 15 and 5 days, respectively. Initial inoculum was distributed equally over all segments. Settings of other parameters are listed in Table 4.1.

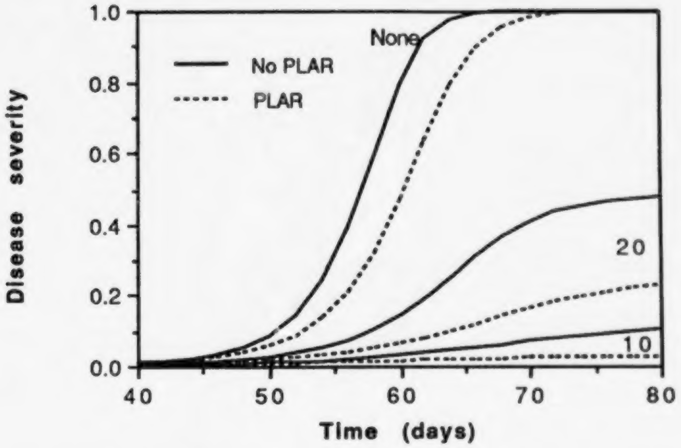

Fig. 4.8. Effect of susceptibility durations of 10 and 20 d (SUSPER) and plant age resistance (PLAR) on simulated disease progress. For SUSPER = None, all growth increments were fully susceptible for the entire simulation. Settings of other parameters are listed in Table 4.1.

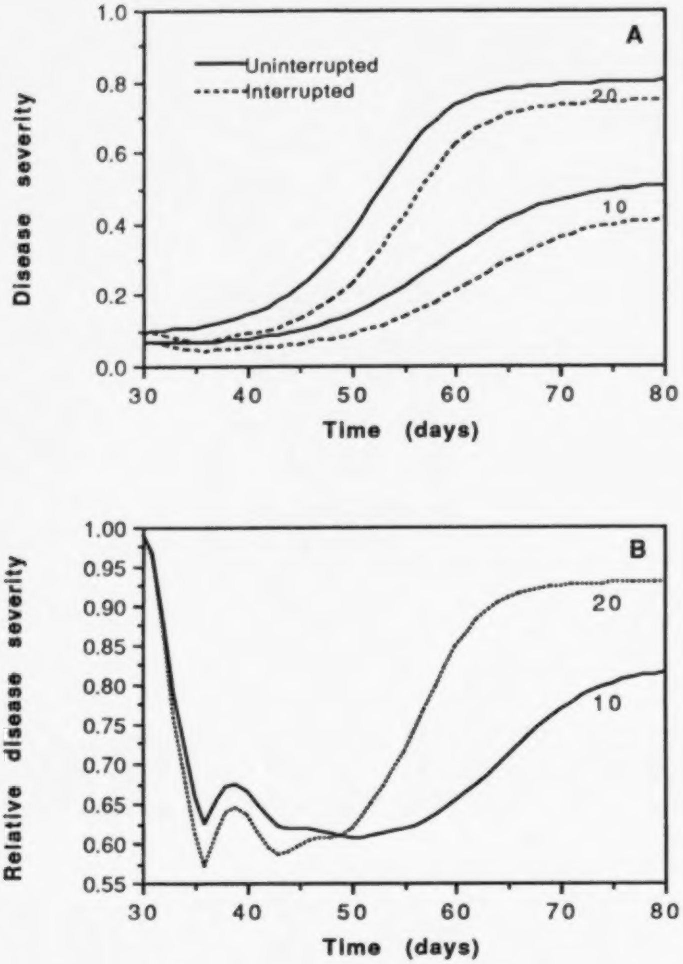

Fig. 4.9. Effect of susceptibility duration (SUSPER) and an unfavorable period of 5 days for infection on simulated disease progress (A) and relative disease severity (B). The relative disease severity is the proportion of the interrupted to the uninterrupted epidemic. The interruption in infection started on day 29. Initial inoculum was distributed equally over all segments. Settings of other parameters are listed in Table 4.1.

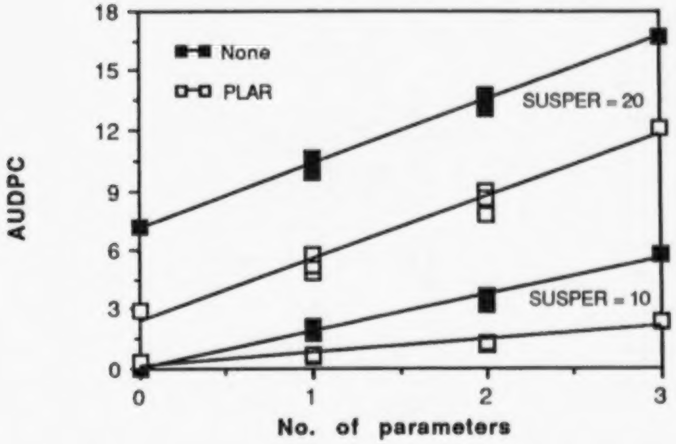

Fig. 4.10. Effect of increasing susceptibility duration (SUSPER), maximum lesion size (Z_{MAX}), and infection efficiency (IE) , with and without plant age resistance (PLAR), on area under the disease progress curves. Initially, each parameter was increased by 20%. then the three combinations of two parameters were increased by 20% and finally, all three parameters were increased simultaneously, each by 20%. Initial inoculum was distributed equally over all segments. Settings of other parameters are listed in Table 4.1.

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Chapter 5. Effect of leaf blast and drought stress on root growth in rice

5.1 Introduction

Blast disease, caused by *Magnaporthe grisea* (Herbert) Barr, is one of most important diseases of rice and is highly destructive in lowland rice in temperate and subtropical Asia and in upland rice in Asia, Africa and Latin America. In rainfed lowland and upland rice, the disease is strongly influenced by drought (Gill and Bonman 1989).

By reducing radiation use efficiency and net photosynthesis, blast on the foliage reduces the production of new healthy leaf area, and therefore shoot growth and yield (Bastiaans, 1993). Less is known about the effects of blast on root growth, although it has been theorized that the disease would reduce allocation of photosynthates to the roots. Drought stress, which reduces root growth (Thangaraj et al. 1990), predisposes rice to infection. Therefore, it is possible that blast and reduced root growth are mutually reinforcing with decreased root growth resulting from infection contributing to even greater levels of disease.

Because foliar pathogens alter root function, nutrient and water uptake would be expected to change as a consequence of blast. This in turn would be expected to cause changes in the rhizosphere, including pH changes. If pH changes in the nutrient solution occurred quickly in response to infection, it is possible that there might be a correlation between tolerance to disease and those pH changes. If so, pH changes in the nutrient solution of hydroponically grown rice could be used as a criterion for selecting tolerant plants.

In this paper, I examined the effects of rice blast on root production in nutrient solution and on root production in soil culture. In addition, I report on the effects of mild drought stress and leaf blast on plant growth.

5.2 Materials and Methods

All experiments were conducted in a greenhouse at the International Rice Research Institute (IRRI), Los Banos, Philippines. The study was undertaken in two parts. In the first part, the effect of blast on root growth in nutrient solution was studied. In the second part, soil culture was used.

General methods for experiments with nutrient solution. Nutrient concentrations were those of Osuna-Canizalez (1991). Seeds were surfaced sterilized for 1 min in a 0.03% sodium hypochlorite solution. After rinsing, seeds were placed on a double layer of cheesecloth and slightly submerged in half-strength nutrient solution for seven days. Each experimental unit consisted of forty uniform seedlings that were placed in a styrofoam support and held in place with rubber foam. Each styrofoam support was placed on a tray containing 6 L of full strength nutrient solution. The pH of the solution was monitored with a pH meter and adjusted to 5.5 with NaOH. Adjustments were done daily unless noted otherwise. Nutrient solutions were changed weekly.

Inoculum preparation and inoculation. *Magnaporthe grisea* isolate PO6-6, obtained from the Entomology and Plant Pathology Division, IRRI, was increased by the method of Bonman et al. (1986). Conidial concentrations were determined by a haemocytometer, and then adjusted to twice the desired concentration before diluting with an equal volume of 2% gelatin. The control consisted of 1% gelatin. All replicates of a given treatment were sprayed at ca. 1700 h by the method of Bonman et al. (1986). Trays were placed into dew chambers for 16 hours, with complete blocks occupying different shelves within a chamber and/or different chambers, before being transferred to a an incubation room,

Effect of blast on plant growth and final pH of the nutrient solution. The experimental design was a split plot. Main plots consisted of three trays of 21 day old plants of cultivar IR50 in each of four blocks. Three inoculum concentrations (0, 25, and 50 x 10^3 conidia/mL) were randomly assigned to the three trays per block. Subplots consisted of repeated measures made on each tray, 4, 7, 10 and 13 days after inoculation. At each time, five plants were selected from each tray, assessed for disease severity by estimating total dead leaf area for the entire plant, and separated into shoot and roots. The plant parts were oven dried at 50 C for 2 days and weighed. On the final assessment date, the pH of the nutrient solution was recorded, and the nutrient solution, approximately 4 L at the end of the experiment, was sampled and analyzed by the Soil Testing Lab at IRRI.

Effect of cultivar and blast on plant growth and daily pH change of the nutrient solution. The isolate PO6-6 and three lowland cultivars IR50, IR64 and IR68, were used. IR50 and IR64 are compatible with isolate PO6-6 whereas IR68 is incompatible. IR50 was considered to have lower partial resistance than IR64 (Yeh and Bonman 1986, Bonman et al. 1988). Plants were inoculated with suspensions containing (0 or 50 x 10^3 conidia/ml) 14 days after transplanting. There were two blocks containing each of the six cultivar by inoculum density treatments in a randomized complete block design. The pH of the nutrient solution was monitored and adjusted to pH 5.5 twice daily at 800 and 1600 hours. Four, 8, 12 and 20 days after inoculation, five plants were selected, separated into shoots and roots, dried and weighed.

Disease severity was assessed for each sampled plant. Data for pH, plant weight, and disease severity were analyzed

The above experiment was repeated to determine if there was a correlation between pH and root oxidation, which has been associated with root respiration (Ando et al, 1980). The pH was monitored on a daily basis. and five plants were dried and weighed 4 and 8 days after inoculation. Because there was less disease in the second experiment, lesion numbers per tray, totalled over susceptible- and resistant-types, were analyzed rather than disease severity. Ten days after inoculation, root oxidation was determined by the method of Ando et al. (1988). Intact roots were pre-incubated in 50 ml of 20 ppm α-naphthylamine (α-NA) for 10 minutes to correct for the initial absorption of α-NA. The roots were transferred to another 50 ml of 20 ppm α-NA and incubated for 3 hours at room temperature. A 2 ml aliquot of the α-NA solution was then sampled and reacted with 10 ml of 0.1% sulfanilic acid in 3% acetic acid and then with 2 ml of 50 ppm $NaNO_2$. The total volume was adjusted to 25 ml and the absorbance of the colored solution was determined at 530 nm. At the same time, different concentrations of α-NA were subjected to the sulfamide and nitrite reactions to produce a standard curve. Roots were oven dried at 50 C for 48 h. The decrease in α-NA during incubation was calculated as the amount of α-NA oxidized/3 hour/g dry root weight.

Effect of plant age and blast on growth of IR50 in soil culture. The experimental design was a randomized complete block, with four blocks and 24 treatments corresponding to two inoculum levels, four seeding dates and three assessment dates. Twenty-four pots of Maahas silty clay-loam soil (Typic Hapludoll) obtained from an IRRI upland site, were seeded with 12 seeds per pot on each of four consecutive days. One week later, the plants were thinned to eight per pot. Plants were inoculated when the sixth leaves of most plants in pots of the first seeding were fully expanded. As a consequence, the average plant ages associated with the four planting dates were 5.125, 5.275. 5.625, and 5.875. At that time, the sixth leaves of the younger plants were at various stages of expansion. One half of the plants from each seeding date were inoculated with a spore suspension of 50 X 10^3 conidia/mL in 1% gelatin, whereas the remaining plants were sprayed with 1% gelatin. After inoculation, plants were placed in a dew chamber for 16 hours, moved to a high humidity room for 4 days and finally placed in a plastic-lined wooden frame for the duration of the experiment. Ten, 17 and 24 days after inoculation, pots from each treatment x replicate combination were selected, and disease was assessed on the fourth to sixth leaves. Plants from each pot were separated into shoots and roots, oven dried and weighed.

Effect of inoculation and post-inoculation water stress on disease progression, soil moisture and plant growth. The experiment was a full factorial with four sampling times (5, 10, 15 and 22 days after inoculation), three inoculum densities (0, 25 and 50 x 10^3 conidia/mL) and two stress levels (saturation and continuous drying) in a completely randomized design with four replicates. Soil was placed into pots having a drainage hole on the side near the base. Eight pre-soaked seeds of IR50 were sown. The plants were grown aerobically and 21 days after seeding, thinned to four uniform plants/pot.

Beginning 1 day after inoculation, pots receiving the saturation treatment were placed in a cart containing water whereas those receiving the continuous drying treatment were placed in a cart without water. Four days after inoculation, the carts were placed in a plastic lined shelter outside the greenhouse for the duration of the sampling period. Pots were weighed on a daily basis. Cart positions were interchanged and pots moved around the tray after weighing to minimize environmental variation in the shelter. Sixteen days after inoculation, imposition of drought was interrupted by a rainstorm which damaged the shelter and soaked the pots.

At each sampling, plants were assessed for disease, leaf area measurements were obtained and shoots were placed into bags for dry matter determination. The pots were placed into a freezer for 4 hours and then the soil was removed and cut into top, middle and bottom segments 5 cm thick. Roots were carefully extracted and placed in the freezer until root length determinations were made. Root lengths for each segment were determined using an automatic root length meter. Root length density was calculated as the ratio of root length to soil segment volume. The roots from the three segments were bulked and the root dry weight was determined. Specific root length was calculated as the ratio of total root length to total root mass.

Only the last three sampling times for plant growth data were included in the analysis. The data from the first sampling time was lost.

Data Analysis. All data were subjected to analysis of variance and/or regression analyses following appropriate transformations where required. Leaf positions were treated as repeated measures.

5.3 Results

Effect of blast on plant growth and final pH of the nutrient solution. Disease severity increased over time (Fig. 5.1A) as a consequence of lesion enlargement, collar blast which caused entire leaves to die, and leaf chlorosis and senescence. The higher inoculum dose resulted in more disease, and a different shaped disease progress curve, than the lower inoculum dose.

Both shoot and root dry weight increased curvilinearly with time for all inoculation levels (Fig. 5.1B). Shoot weights, but not root weights, were significantly less on diseased than control plants. However, neither shoot nor root weights changed with the increase in inoculum from 25 to 50 x 10^3 spores/ml. Furthermore, there was no significant difference between controls and diseased plants in root:shoot ratios.

The nutrient solution pH measured on the last sampling date increased with diseased leaf area (Fig. 5.1C). Nutrient solution pH was weakly correlated with final shoot dry weight ($r=0.56$, $P>0.05$) and root dry weight ($r=0.36$, $P>0.10$) and strongly correlated with disease severity ($r= -0.88$, $P<0.001$) and the joint effects of shoot dry weight and percentage healthy leaf area, which reflects the total amount of healthy leaf area ($r=0.79$, $P<0.001$).

After 13 days, there was more potassium and phosphorus, and less iron and sodium in nutrient solutions from diseased than healthy plants (Table 5.2). There were no differences for nitrogen, sulfur, calcium, manganese, zinc, or copper.

Effect of cultivar and blast on plant growth and daily pH change of the nutrient solution. - Experiment 1. Disease severities at the end of the experiment were 19.5%, 11.2%, 0.6% for IR64, IR50 and IR68, respectively. On IR68, there were no susceptible-type lesions; only brown specks, typical of resistant-type lesions, were observed. On IR50 and IR64, lesions were largely of the susceptible -type; lesions, senescence, and leaf death due to collar blast contributed to diseased leaf area on these two cultivars.

Blast significantly depressed the average root growth of IR50 and IR64. It also significantly depressed root growth of IR68 over the first 12 days ($P < 0.10$) but not at 20 days presumably because growth of the uninoculated plants was affected by low pH of the nutrient solution (Fig. 5.2). Similarly, the average shoot weights of IR50 and IR64 were depressed by inoculation, but the shape of the shoot growth curve of IR68 was changed from curvilinear to linear (Fig. 5.2). There was no effect of inoculation on the root:shoot ratio.

There were large day-t0-day fluctuations in pH (Fig. 5.3A) presumably because overcast conditions occurred occasionally during the monitoring period. To account for initial differences in pH among trays, the pH 16 hours after inoculation (time of the last pH adjustment) was subtracted from the pH readings at all subsequent times. Regardless of cultivar, the pH of the nutrient solution was significantly greater for inoculated than uninoculated plants ($P<0.01$), the effect of inoculation on pH was not clearly associated with cultivar resistance being greater for IR64 than IR50 and IR68. The effect of inoculation over all cultivars was significant at most times beginning on the fourth day after inoculation. Differences between inoculated and uninoculated plants of IR50 and IR64, but not IR68 increased linearly over time (Fig. 5.3B). The slope for IR64 was significantly greater than IR50.

Effect of cultivar and inoculum dose on disease, plant growth and daily pH change of the nutrient solution. - Experiment 2. Disease severity was less in the second experiment than the first experiment and did not exceed 10% on any plant of any cultivar. Eight days after inoculation, the number of lesions per plant totalled over resistant- and susceptible-types, did not differ significantly between IR50 and IR64 (Table 3). There were significantly ($P<0.001$) fewer lesions on IR68 and they were all of the resistant type.

In general, inoculation did not significantly affect plant growth. Although shoot weight and root weight were less on diseased than healthy plants (Table 5.3), the differences were not significant. There were no effects of inoculation on the root:shoot ratio. The number of roots/plant of inoculated plants were 89, 88 and 98% of controls for IR50, IR64 and IR68, respectively (Table 5.3), but these differences were not significant.

The pH of the nutrient solution was not significantly altered by inoculation or cultivar. Eight days after inoculation, the differences in pH of the nutrient solution between inoculated and control plants were 0.27, 0.15, and -0.08 for IR50, IR64 and IR68, respectively. Oxidation of α-NA was significantly reduced by inoculation ($P < 0.01$). Oxidation of α-NA by infected plants was 72, 69 and 82% that of control plants for cultivars IR50, IR64 and IR68, respectively (Table 5.3).

Effects of plant age and disease on plant growth of IR50 in soil culture. No secondary infection occurred as all lesions were confined to the 6th or lower leaves. Nevertheless, disease severity increased over time on all inoculated plants. Differences in disease severity among seeding dates and assessment times were significant only on the 6th leaf, possibly due to the high coefficient of variation (84-127%). Disease severity was greatest on the youngest (last seeding date) plants, although there were no significant linear or quadratic trends in disease severity with seeding date on the sixth ($F<1$). Both the linear and quadratic components of the sum of squares for harvest dates of that leaf were significant.

For control plants, both shoot and root dry weights increased linearly with plant age (Fig. 5.4). In contrast, for inoculated plants, the shapes were curvilinear. In case of shoot curves, the degree of curvature increased with the time after inoculation at which the plants were assessed (Fig. 5.4A). In contrast for roots, although similiar trends were found, the increase in curvature on inoculated plants with assessment time was not significant (Fig. 5.4B).

Effect of inoculation and post-inoculation water stress on disease progression, soil moisture and plant growth. Disease severity increased over time for the duration of the experiment (Fig. 5.5). After the 5-day post-inoculation disease assessment, the increase in severity was largely due to leaf death from collar blast or the senescence of tissues distal to lesions near the collar. There was no significant difference in disease severity between plants inoculated with 25 or 50 x 10^3 spores/mL. Post-inoculation drought stress decreased disease severity on inoculated plants slightly, but this difference was not significant.

Among plants not subjected to water stress, there was no significant difference in moisture content between inoculated and control treatments (Fig. 5.6). In contrast, on water stressed plants soil moisture content was greater for inoculated than uninoculated treatments. Following the interruption in water stress which occurred 16 days after inoculation, the rate of water loss was greater than before the occurrence of the storm and water loss was slightly less for inoculated than uninoculated plants. There were no apparent symptoms of drought stress, although portions of leaves distal to lesions on or near the central vein or leaf collar were leaf rolled, senesced and dead on both unstressed and stressed plants.

The linear rates of dry matter production were significantly less for leaves ($P < 0.01$), stems ($P < 0.01$) and roots ($P < 0.01$) from inoculated plants than controls (Fig. 5.7). Plant growth was affected less by drought stress than inoculation; the sum of squares for the combined effects of inoculation and inoculation with time interaction was greater than the combined effects of drought stress and stress with time interaction by factors of 6, 3 and 894 for leaves, stems and roots, respectively. Although drought stress decreased mean stem ($P < 0.05$) and leaf ($P < 0.01$) weights, the linear rates of weight increase were not significantly different. The effect of drought was independent of inoculation; neither the interaction of drought and inoculation nor the three way interaction between drought, inoculation and time were significant.

The root:shoot ratio increased linearly for controls whereas the ratio increased curvilinearly for inoculated plants (Fig. 5.8). At 22 days after inoculation, the root:shoot ratio was less for inoculated plants than controls ($P < 0.05$). A similar response was observed for root:stem and root:leaf ratios but differences were significant only for the latter. Drought stress had the opposite effect of disease: root:shoot, root:stem and root:leaf leaf ratios were greater for drought-stressed than control plants

($P < 0.05$ - $P < 0.10$) and differences remained relatively constant over time. As before, there was neither an interaction between disease and drought, nor a three way interaction with time.

Root length density varied with depth, inoculation and stress. Root length density decreased curvilinearly ($P<0.001$) with depth (Fig. 5.9). Because of heterogenity of variances, the top profile was analyzed seperately from the middle and bottom profiles. Differences in root length between healthy and diseased plants increased linearly over time in the top section ($P<0.001$), Fig. 5.9A) but root length density did not vary significantly over time on drought-stressed plants. However, in the middle and bottom sections of the profile, the increase in root length depended on both inoculation and drought stress (Fig. 5.9B-9C). In the middle profile, the root length of uninoculated stressed plants was greater than uninoculated unstressed plants 15 days after inoculation whereas at 22 days after inoculation, the root length was greater for uninoculated unstressed plants than uninoculated stressed plants (Fig. 5.9B) These differences between stressed and unstressed plants were negated by inoculation. In the bottom profile, root lengths were greater for uninoculated stressed plants than uninoculated unstressed plants at both 15 and 22 days after inoculation (Fig. 5.9C). Once again, these differences were negated by inoculation.

Specific root length decreased linearly with time ($P<0.01$, Fig. 5.10). Although the specific root length was greater on inoculated than healthy plants, differences were not significant. At 10 days after inoculation, specific root length density was less for stressed than unstressed plants. However, specific root length decreased rapidly on unstressed plants; at 15 and 22 days after inoculation, specific root lengths of stressed and unstressed plants were not significantly different.

5.4 Discussion

Although patterns of plant part growth were similiar, root growth was less affected by inoculation than shoot growth (Figs. 5.1B, 5.2, 5.4). The lower leaves normally provide more assimilates to the roots than to other parts of the plant (Tanaka, 1958). However, if the upper leaves are not functioning properly due to disease, insects or leaf death, the lower leaves may provide additional assimilates to the upper leaves at the expense of the roots (Yoshino 1981). The differences in pH between solutions bathing inoculated and uninoculated plants could reflect, in part, this altering of assimilate sinks. Similarly, root oxidation, which is a measure of root respiration, was lower in inoculated plants, perhaps because of a reduction in the amount of assimilates reaching the roots or disruption of the flow of oxygen from the leaves to the roots through the aerenchyma.

Major contributors to pH changes in the rhizosphere are the relative rates of cation and anion uptake (Yoshino 1981, Taylor and Foy 1985,), root oxidation (Kirk et al. 1990), uptake of carbon dioxide by the roots (IRRI 1991) and release of organic acids. Uptake of cations is accompanied by extrusion of protons and uptake of anions is accompanied by release of bicarbonate ions and the relative difference in release of protons and carboxyl ions determines the change in pH. In spite of the substantial differences in pH associated with the inoculation treatment, there were no equivalent changes in nutrient uptake. This could reflect the high concentration of ammonium used in these experiments. High uptake rates of NH_4-N increased uptake of some ions, but the mechanism is not clear (Harada et al. 1968, Walters and Ayres 1981). Because plants were grown in nutrient solution, the uptake of nutrients should not be limited by nutrient availability as affected by diffusion nor water availability. The uptake of some nutrients may be reduced by lowering the pH of the medium. In field beans (Vicia faba), proton release and nutrient uptake was reduced at pH 4 and enhanced at pH 7. Further studies are required to determine the relationship of nutrient uptake to the change in pH of the nutrient solution.

Root oxidation is a proton releasing process (Kirk et al. 1990, IRRI 1988). Stoichiometrically,

$$4\ Fe^{++} + O_2 + 10\ H20{=}4Fe(OH)_3 + 8\ H^+$$

It is a process whereby roots in anaerobic conditions are protected from high concentrations of metal ions and hydrogen sulfide. The ions are oxidized to render them insoluble and unavailable for absorption by roots. Root oxidation was the predominant source of proton release in rhizosphere soil and can have profound effects on further uptake of nutrients (IRRI 1990, 1991).

The effect of inoculation on pH could have implications for nutrient uptake. The concentration of iron was lower in the nutrient solution bathing inoculated than uninoculated plants (Table 5.2) This suggests that the concentrations of this element in the plant could increase with disease severity, and therefore the possibilty of iron toxicity. Recent simulations showed that the decrease in rhizosphere pH resulting from Fe^{++} oxidation reduced ammonium ion uptake (IRRI 1991). Nitrogen content was greater on infected than healthy rice near the end of the growing season. This was dilution by the larger biomass of healthy than infected plants (Bastiaans 1993). My results suggest an alternative possibility: disease may have reduced oxidatation, increasing rhizosphere pH and nitrogen uptake. In addition, acidification of the rhizosphere will increase the silica concentration in solution and hence, availability to the roots (IRRI 1991). These results suggests that the partial resistance of the plant may be modified further by the effects of disease on rhizosphere pH.

The pH of the nutrient solution was strongly correlated with disease severity and with an approximate estimate of healthy leaf area but not with root mass. Furthermore, large differences in pH between controls and inoculated plants coincided with symptom expression (Fig. 5.4). This raises the possibility that cultivars that are tolerant of the effects of blast on foliar or leaf function could be selected on the basis of pH differences in nutrient solutions. However, although inoculation affected the pH of the nutrient solution, the change in pH was not correlated with resistance, presumably because pH of the nutrient solution varied with cultivar and the differential response of different cultivars to inoculation.

Although disease severity assessment included increased senescence due to infection, disease severity was greatest on those plants that were youngest at the time of inoculation (Table 5.5) and there was a large reduction in shoot and root weight on these plants (Fig. 5.5). There was no difference in mean disease severity among plants in the older three age classes (Table 5.5). Nevertheless, there was a significant reduction in both shoot and root growth on those plants that were the oldest (age=5.875) at the time of inoculation. This might reflect the differential effects of lesion position on leaf physiology. Lesions on the main vein of the leaf reduced photosynthesis more on leaf parts distal to the lesion than leaves with similar disease severities but without lesions on the main vein (Bastiaans 1993). The relative position of lesions on the leaves is important in determining the effect of disease on crop yield loss (Zadoks and Schein 1979) and similar disease severities at different stages of plant development show different effects on yield loss (Gaunt and Teng 1981). Similar results could be expected for the effects of disease on root function.

Disease severity was not exacerbated by withholding water after inoculation, presumably because drought stress was mild. Although disease severity appeared to increase more rapidly on stressed plants, this apparent interaction was not significant. The level of drought stress during lesion development may not have been sufficient to alter disease progression. Also, disease did not exacerbate the effects of drought stress. Leaf area was reduced, which probably decreased the transpiration rates and conserved soil moisture. However, leaf parts distal to a point of infection showed some signs of water stress, prior to death. Lesions on the main vein could have disrupted the flow of water to leaf parts distal to the lesion. Collar infections induced leaf roll even on well watered plants.

Rice shoot growth is quite sensitive to water deprivation. Decreasing soil moisture increases stomatal and mesophyll resistance to CO_2, thereby reducing carbon assimilation (Dinkuhn et al. 1990), and also inhibits cell expansion, thereby reducing leaf area. In my experiments, plants were exposed to separate drying cycles due to an interruption of drought stress 16 days after inoculation. The seedlings, therefore, experienced only mild drought stress, as indicated by the lack of leaf roll.

The effect of drought stress varied with plant organ. Leaf growth was reduced during the first, but not the second, drought cycle (Fig. 5.7B), perhaps as a consequence of leaf turgor being maintained during the second drought cycle. In contrast, stem growth was reduced during the second drought cycle (Fig. 5.7B), perhaps because of osmotic adjustment, preferential translocation of photosynthate to the roots, or reallocation of stem reserves to the roots. Although stem reserves have been reported to be important in grain filling (Yoshida 1981), it is unknown if these reserves are used for the production of new roots in response to drought stress.

Blast had a greater affect than drought stress on plant growth. This reduction could have resulted from leaf mortality and decreased carbon assimilation which could have reduced leaf area production and stem weight increment. Evapotranspiration from pots with inoculated plants may have been reduced by the reduction in leaf area. The apparent independence of drought stress and inoculation effects on plant growth may have occurred because the drought stress occurred only after most of the lesions had appeared.

Following application of mild drought stress, root growth in the top horizon was inhibited, whereas growth in the bottom horizon was slightly increased. This was somewhat in contrast to previous studies (Cruz et al. 1981, Thangaran and De Datta 1986) in which root growth was inhibited in all depths with the amount of reduction decreasing with depth. The interaction between drought stress and soil depth on root growth may have arisen because of changes in soil strength with soil depth. Upper horizons would be drier and more resistant to root penetration than lower, moisture horizons (Thangaraj and De Datta 1981).

Inoculation also reduced root growth, with the effect occurring in all horizons. In addition, the compensatory changes in root:shoot ratio that often occur with drought stress were inhibited by inoculation (Fig. 5.9). Thus, in a prolonged period of drought, diseased plants could be more prone to drought stress than healthy plants. Subsequently, susceptibility of diseased plants could be increased further, and particularily if a stress threshold is attained. Thus, using cultivars with partial resistance and deep root systems allowing plants to avoid severe drought stress could be recommended for disease management.

In summary, inoculation reduced both shoot and root growth. Although root growth was not as sensitive as shoot growth, root function was affected soon after inoculation. The apppearance of lesions on the foliage was accompanied by reduced proton extrusion into the nutrient solution; root oxidation was also reduced by inoculation. The effect of disease on root growth was dependent on the age of the plant at inoculation. The effects of disease on plant growth were greater than the effects of mild drought stress and roots were particularily sensitive to blast on the foliage.

Table 5.1. Chemical composition of the stock and nutrient solution used in the hydroponic culture experiments.

	Stock solution		Nutrient solution	
Nutrient	mol/m^3	g/L	mmol/m^3	g/L
$(NH_4)_2SO_4$	1071.00	141.520	7000.00	0.92498
$Na_2HPO_4.7H_20$	64.00	17.160	80 00	0.02145
KH_2PO_4	194.00	26.400	240.00	0.03266
K_2SO_4	312.00	54.370	390.00	0.06796
$CaCl_2.2H_20$	798.00	117.320	1000.00	0.14702
$MgSO_4.7H_20$	1314.00	323.860	1645.00	0.40544
$Na_2SiO_3.9H_2O$	500.00	142.030	200.00	0.05681
Fe-EDTA†	100.00	83.400	100.00	0.08341
H_3BoO_3	15.11	0.934	19.00	0.00116
$MnCl_2.4H_2O$	7.58	1.500	9.48	0.00188
H_2MoO_4	0.44	0.071	0.55	0.00009
$ZnSO_4.7H_2O$	0.12	0.035	0.15	0.00004
$CuSO_4.5H_2O$	0.12	0.030	0.15	0.00008

† Fe-EDTA Mixed 89 g EDTA in 900 ml 1 N NaOH and 83.4 g $FeSO_4.7H_2O$ in 1 liter and made to a final volume of 3 liters.

Table 5.2. Proportion of nutrients remaining in the nutrient solution at last sampling after inoculation with three levels of *Magnaporthe grisea*.

	Element			
Conidia/mL ($\times 10^3$)	N	P	K	S
0	0.47	0.53	0.52	0.63
25	0.52	0.58	0.63	0.57
50	0.48	0.60	0.63	0.57
LSD(.05)	0.18	0.05	0.03	0.24
Conidia/mL ($\times 10^3$)	NO_3+NO_2	Ca	Mg	Cu
0	0.50	0.74	0.81	0.34
25	0.45	0.71	0.76	0.41
50	0.47	0.71	0.72	0.59
LSD(.05)	0.08	0.09	0.31	0.39
Conidia/mL ($\times 10^3$)	Fe	Mn	Zn	Na
0	0.80	0.06	0.43	0.67
25	0.68	0.04	0.60	0.54
50	0.66	0.04	0.55	0.53
LSD(.05)	0.06	0.04	0.29	0.04

Table 5.3. Disease severity, shoot and root weights and α–napthylamine (α-NA) oxidation of three cultivars inoculated with two levels of *Magnaporthe grisea*.

		Lesions/ Plant	Shoot Wt (g)	Root Wt(g)	pH	α-NA[‡] oxidation
Cultivar	Dose\DAI[†]	8	8	8	8	10
IR 50	0	0.0	0.98	0.19	3.24	11.62
	50	20.3	0.93	0.18	3.54	8.36
IR 64	0	0.0	0.98	0.18	3.30	11.83
	50	18.2	0.84	0.17	3.45	8.18
IR 68	0	0.0	1.28	0.24	3.32	7.84
	50	1.4	1.02	0.22	3.24	6.46

[†] Conidia/mL \ Days after inoculation when sampled.

[‡] Ng of α-NA/g root/hr. The F-value for main effect of Inoculation was significant at $P < 0.01$. The F - value for the inoculation with cultivar interaction was not significant ($P=0.76$) and sum of squares was pooled with error.

Table 5.4. Disease severity on the fourth, fifth and sixth leaves. Seeds were planted on four consecutive days and sampled at three times following inoculation.

		Leaf Position		
Factor	Level	4	5	6
Seed date	0	0..09	0.08	0.09
	1	0.08	0.10	0.09
	2	0.09	0.11	0.09
	3	0.17	0.15	0.14
Time	10	0.03	0.03	0.06
	17	0.18	0.14	0.14
	24	0.14	0.14	0.11

Fig. 5.1. Disease severity on IR50 inoculated with Magnaporthe grisea at 25×10^3 (O) and 50×10^3 (Δ) spores/mL (A). The inoculation with time interaction was significant at the 0.05 level. The vertical line represents the standard error of the difference between disease severity means at a given time. Shoot (O) and root (□) dry weights at various days after inoculation (DAI) for uninoculated (——) and inoculated (-----) plants (B). There was no significant difference in shoot and root weights for plants inoculated with 25×10^3 and 25×10^3 spores/ml and therefore, these data were combined. The pH of the nutrient solution as a function of final disease severity (C). The curvilinear trend was significant at $P < 0.05$.

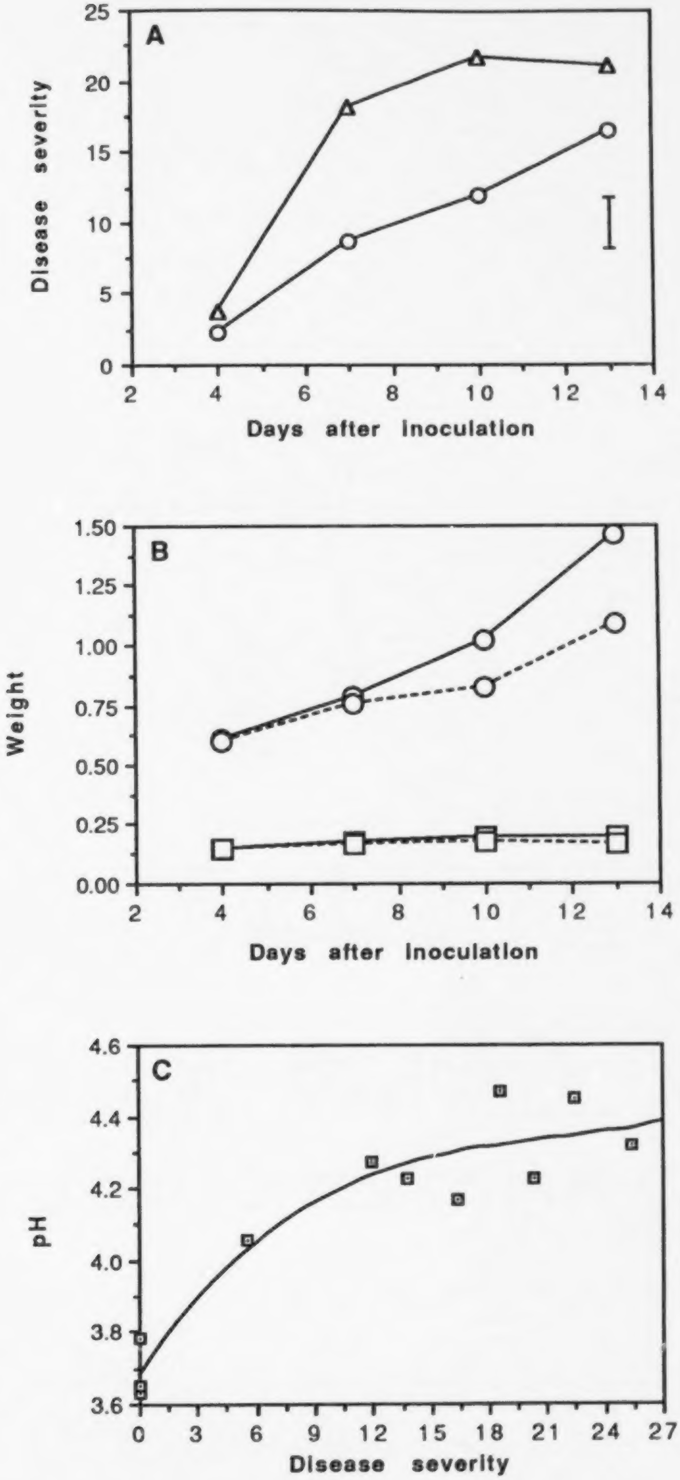
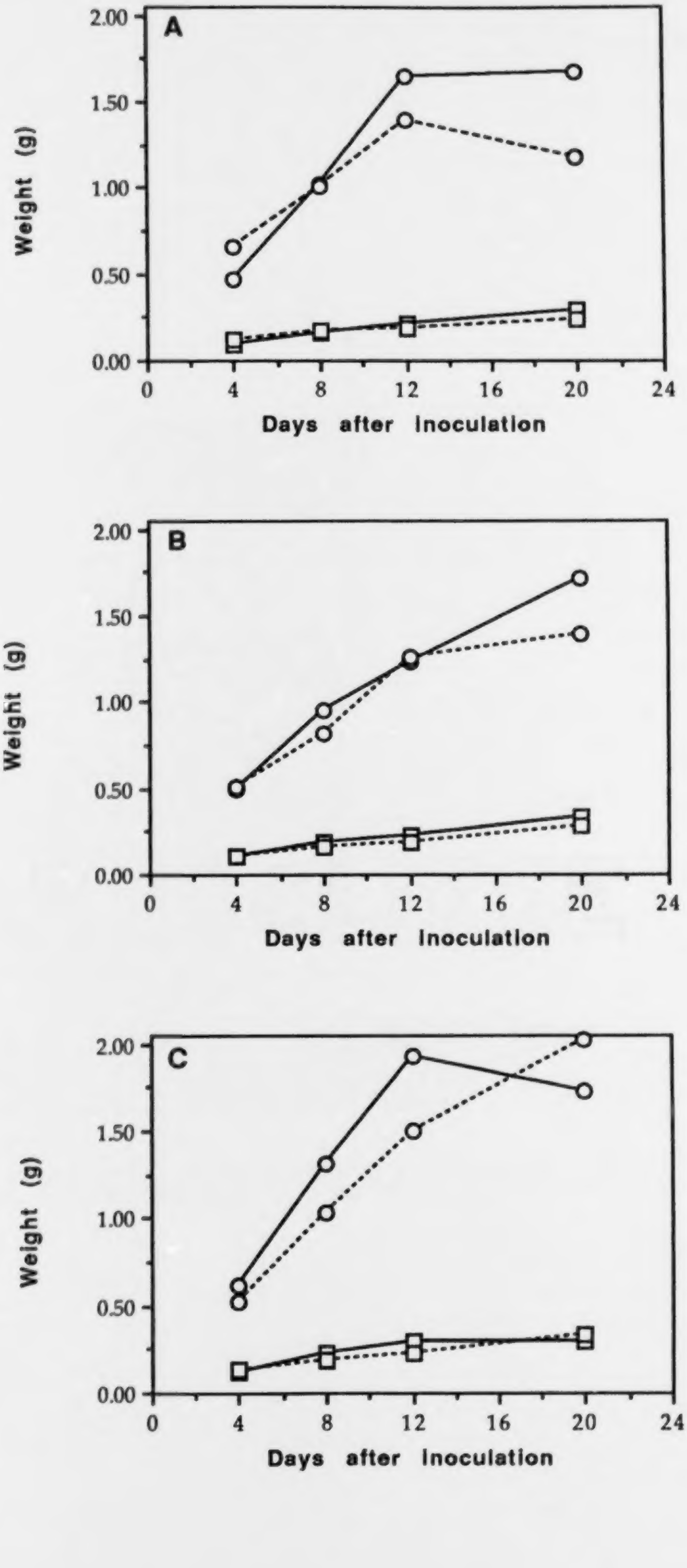

A
Disease severity
0
5
10
15
20
25
Days after inoculation
2
4
6
8
10
12
14
B
Weight
0.00
0.25
0.50
0.75
1.00
1.25
1.50
Days after inoculation
2
4
6
8
10
12
14
C
pH
3.6
3.8
4.0
4.2
4.4
4.6
Disease severity
0
3
6
9
12
15
18
21
24
27

Fig. 5.2. Shoot (O) and root weights (□) of uninoculated (——) and inoculated (----) plants for cultivars IR50 (A), IR64 (B) and IR68 (C). The F-value for the interaction of inoculation with cultivar by assessment time on shoot weight was significant at $P < 0.01$. Shoot growth was curvilinear for IR50 and linear for IR64 and was significantly reduced by inoculation on IR50 ($P<0.001$) and IR64 ($P<0.10$). For IR68, shoot growth was curvilinear for uninoculated plants and linear for inoculated plants. The F-value for the interaction of inoculation x cultivar x assessment time on root weight was significant at the 0.01 level. The inoculation with time (linear) contrast was signifcant for IR50 ($P<0.01$) and IR64 ($P<0.10$) and the inoculation with time (quadratic) contrast for IR68 was significant at $P < 0.001$.


A
B
C
Weight (g)
2.00
1.50
1.00
0.50
0.00
0
4
8
12
16
20
24
Days after inoculation

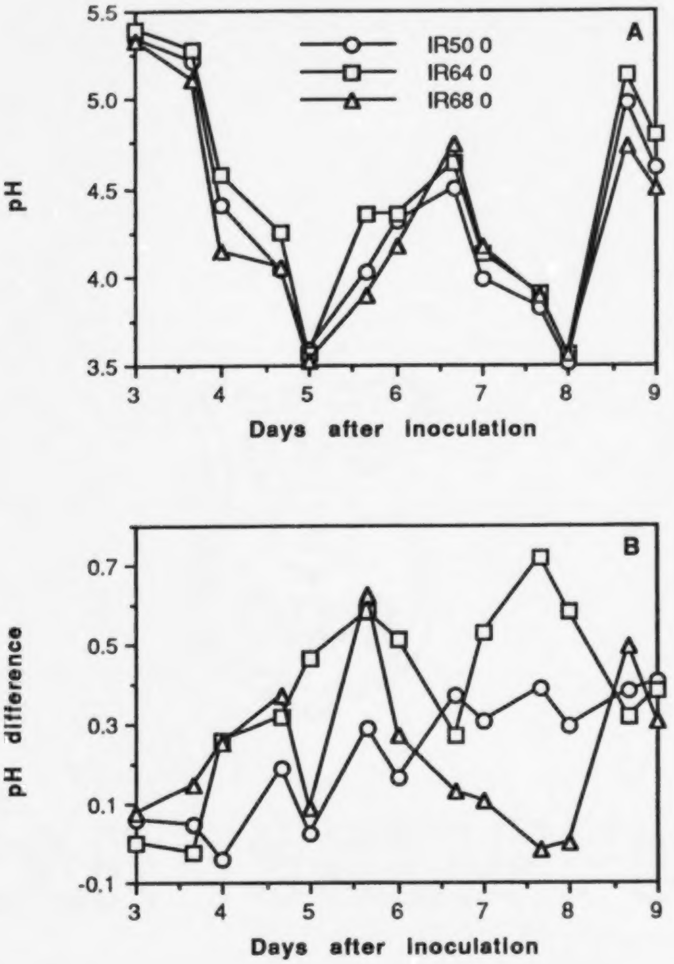


Fig. 5.3. The pH of nutrient solutions for uninoculated plants of three cultivars, IR50 (O). IR64 (□) and IR68 (Δ). [A] and the difference in pH between uninoculated and inoculated plants [B].respectively. The F-values for the time main effect and the cultivar with inoculation interaction were significant at $P<0.001$ and $P<0.05$, respectively. Differences between uninoculated and inoculated plants increased linearily for IR50 and IR64 but not IR68. The slope for IR64 was greater than IR50 ($P<0.05$).

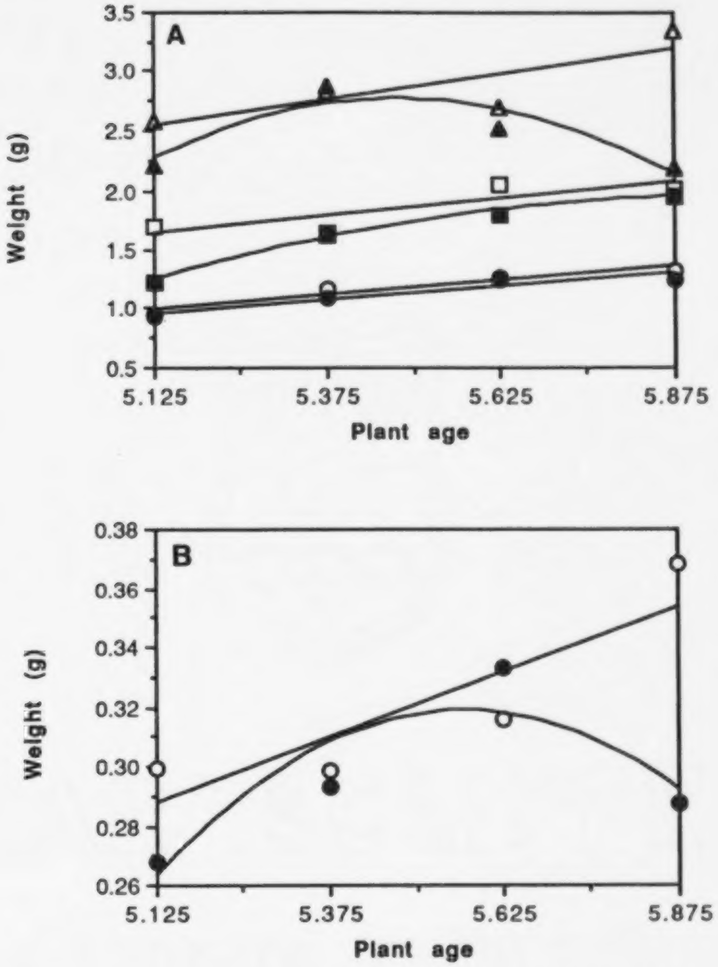


Fig. 5.4. Shoot weights of uninoculated (open symbols) and inoculated (closed symbols) plants of different ages at 10 (O), 17 (□) and 24 (Δ) days after inoculation. (A). The F value for the interaction of seeding date with inoculation by time for shoot weight was significant at the 0.05 level. Root weights averaged over three sampling dates (B) for plants of different ages. The F values for the inoculation with seeding date and seeding date with assessment time interactions for root weight were significant at the 0.05 and 0.01 levels, respectively.

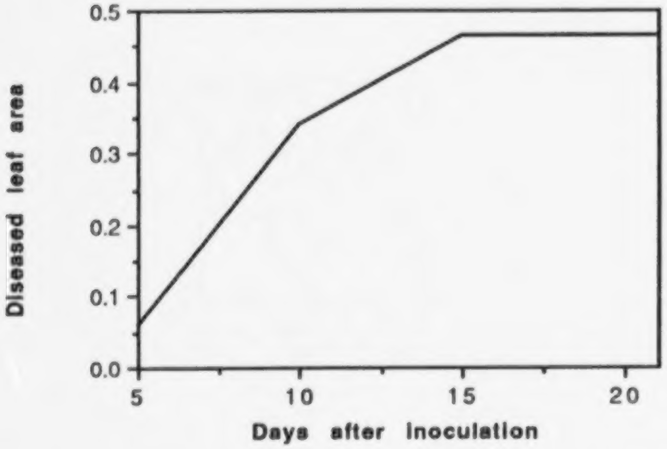


Fig. 5.5. Disease progress on the main tiller of inoculated plants. No significant differences were observed for disease severity between inoculum levels.

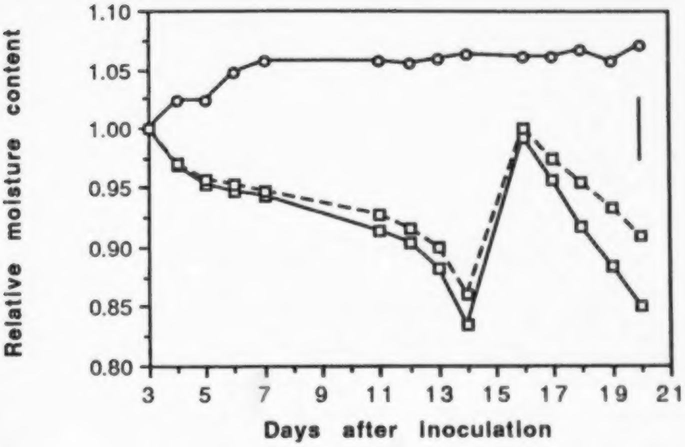


Fig. 5.6. Soil moisture content, relative to 3 days after inoculation, for uninoculated (——) and Inoculated (-----) plants growing in saturated (O) or continuously drying soil (□). The sudden increase in soil moisture content on day 16 occurred as a result of damages sustained in a tropical storm. Data for plants inoculated with 25 and 50 X10^3 conidia/mL were combined in plots for inoculated plants in continously drying soil, data for plants inoculated with 0, 25 and 50 x 10^3 conidia/mL were conbined in the plot of saturated soil. The bar represents the LSD between the moisture content of uninoculated and inoculated treatments at a given time at P = 0.05.

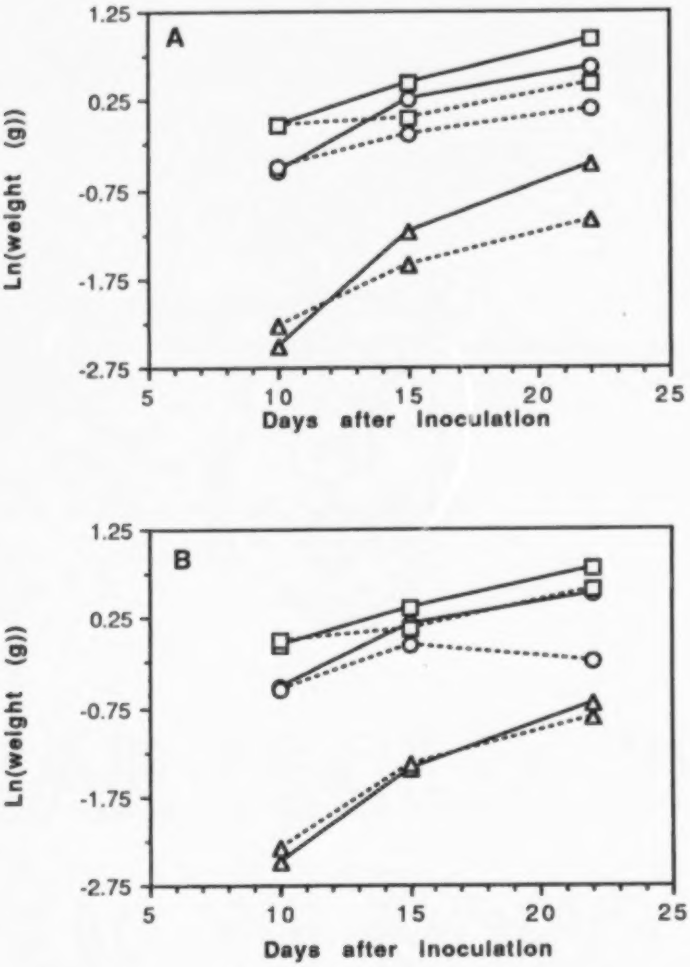


Fig. 5.7. Effect of inoculation (A) and drought stress (B) on dry weights of stems (O), leaves (□) and roots (Δ). In each graph, stress, biotic or abiotic, is indicated by the dashed line. A. The F values for the interaction of inoculation X time (linear) for leaves, stems and roots were significant at P<0.001, 0.05 and 0.01, respectively. B. The main effects of drought stress, but no interactions, for leaves and stems were significant at P<0.05.

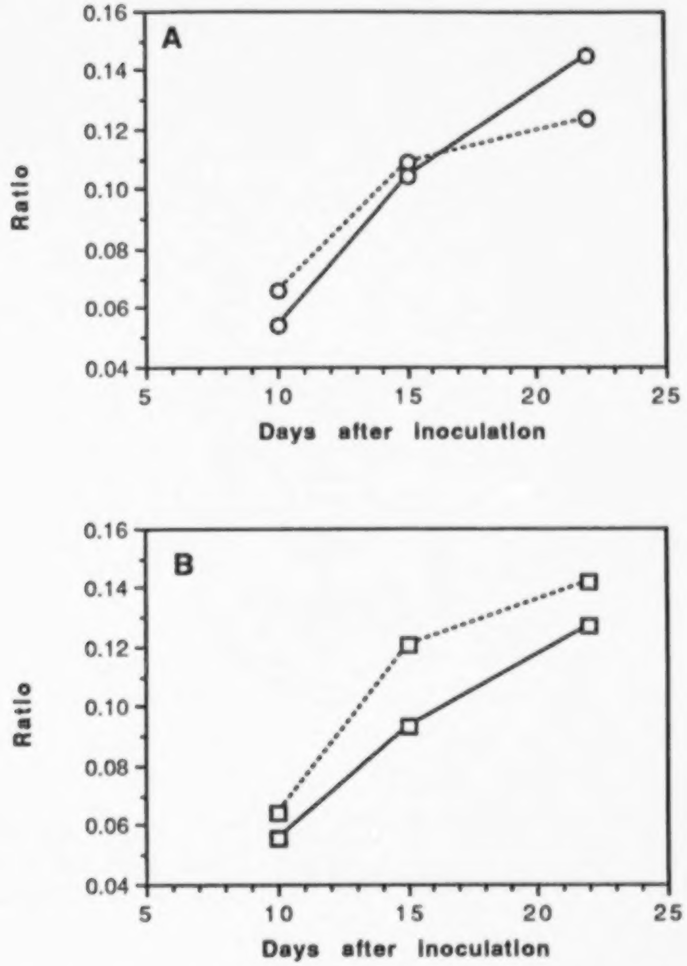
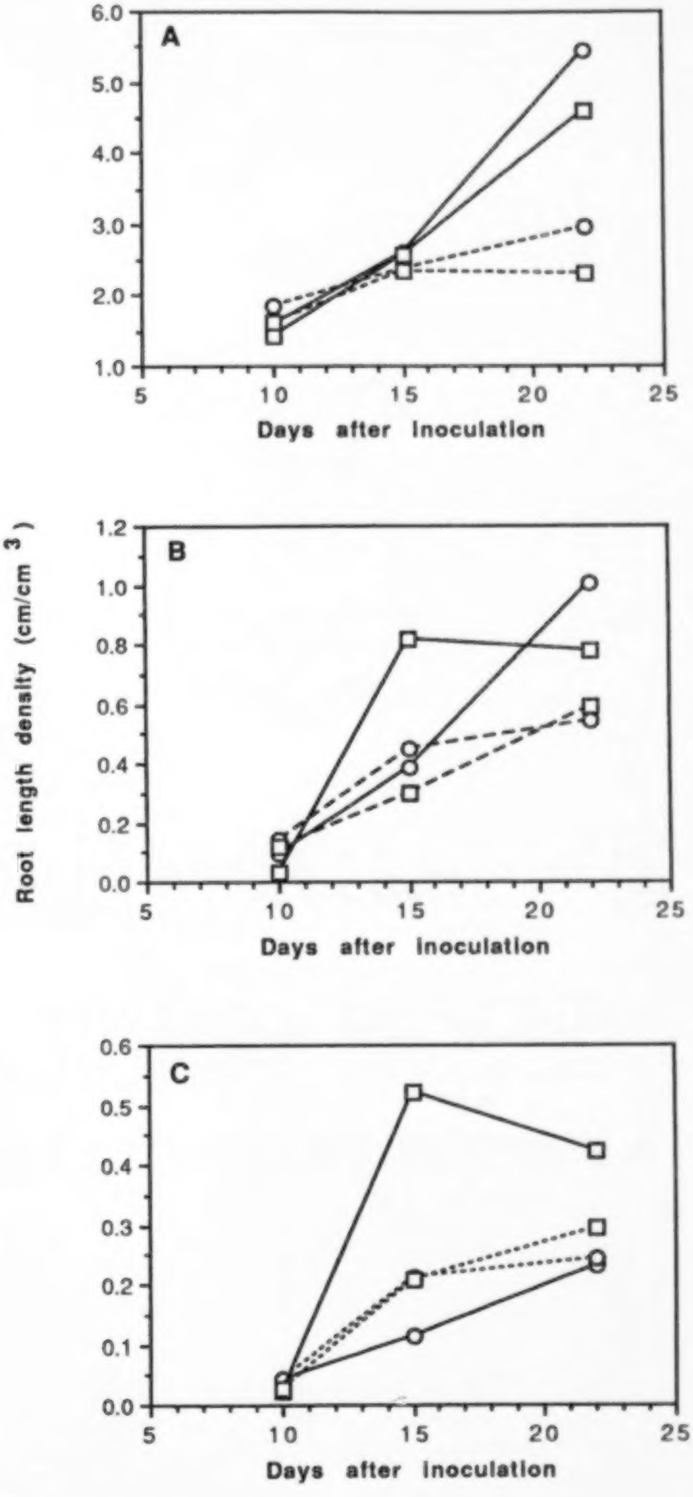


Fig. 5.8. Root:shoot ratios as affected by inoculation (A) and drought stress (B). In each figure, stress, biotic or abiotic is indicated by the dashed line. F-values for the main effects of assessment time and drought stress were significant at $P < 0.001$ and 0.05, respectively. Neither the inoculation effect nor any interaction effect were significant.

Fig. 5.9. Root length density at depths of 0-5 cm (A) 5-10 cm (B) and 10-15 cm (C) for uninoculated (——) and inoculated (-----) plants subjected to drought stress (No drought stress (O), drought stress (□)) observed 10,15 and 22 days after inoculation. A. The inoculation with assessment time interaction was significant at the 0.001 level. There was no significant effect of drought stress. B-C. Wilks Lambda for the interaction of depth x inoculation x drought stress by assessment time was significant at $P < 0.05$. Note the change in scale with depth.


A
6.0
5.0
4.0
3.0
2.0
1.0
5
10
15
20
25
Days after inoculation
B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
5
10
15
20
25
Days after inoculation
Root length density (cm/cm 3)
C
0.6
0.5
0.4
0.3
0.2
0.1
0.0
5
10
15
20
25
Days after inoculation

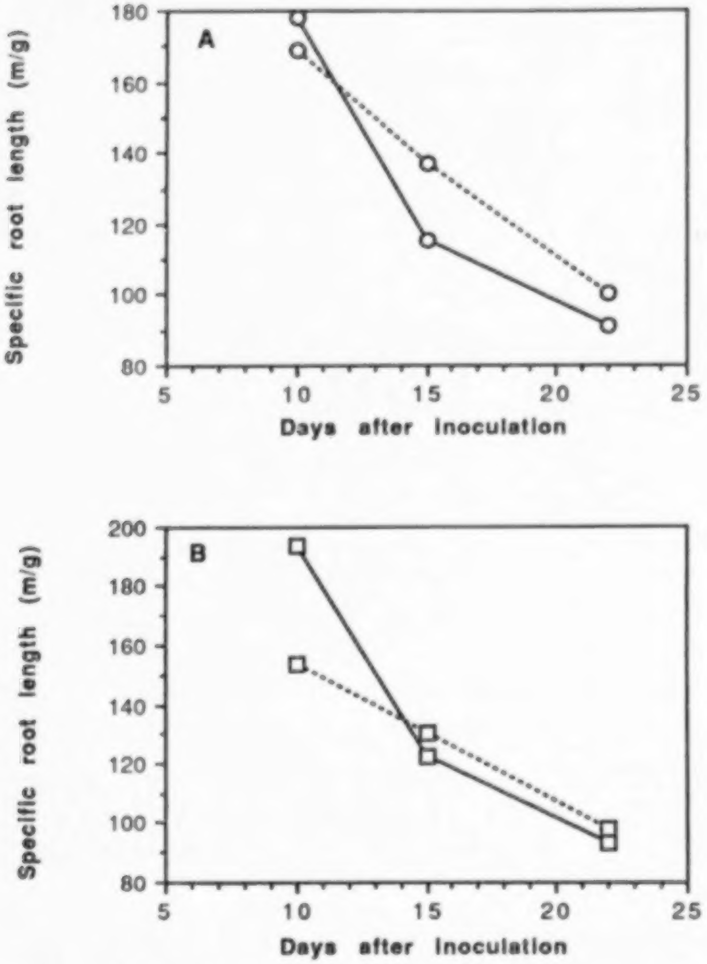


Fig. 5.10. Specific root length density as affected by inoculation (A) and drought stress (B). In each figure, stress, biotic or abiotic is indicated by the dashed line. F-value for the main effect of drought stress was significant at the 0.05 levels. Neither the inoculation effect nor any interaction effect were significant.

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Chapter 6. General Discussion

When disease progress on one cultivar, which is susceptible to a particular race of a pathogen, is reduced compared to another cultivar which is susceptible to the same race, the first cultivar has partial resistance to the disease (Parlevliet 1979). Partial resistance has often been correlated with one or a combination of reduced infection efficiency, longer latent periods, sporulation capacity and longer infectious periods. In the *Oryza sativa* L. - *Pyricularia oryzae* Hebert (Barr), the infection efficiency decreased with tissue age (Kahn and Libby 1959, Volk et al. 1959). Partial resistance was related to the speed at which leaves became resistant with leaf age (Roumen 1993).

Partial resistance can be modified by plant nutrition and environmental conditions. For example, nitrogen application was important in increasing the infection efficiency of Puccinia graminis on wheat, but overall rankings of the cultivars were similar at all nitrogen levels (Shaner et al. 1978). Application of nitrogen one day before inoculation increased blast lesion number an average of 150% on seven cultivars of rice, but again the ranking was similar to unfertilized plants (De Boef 1989). Other components of partial resistance were also affected in the latter study. Although nitrogen application was associated with mean lesion size, the effect was greater on the maximum lesion size.

Pre-inoculation drought stress was important in increasing plant susceptibility to infection in both greenhouse and field studies (Gill and Bonman 1988, Bonman et al. 1988, Table 2.3, Figs. 2.2-2.6). Furthermore, effects of drought stress varied with cultivar (Gill and Bonman 1988, Table 2.3), but in the study by Gill and Bonman, rankings were similiar. This could be related to the wider range of resistance used. In this study, there was an interaction of cultivar and drought stress on the relative infection efficiency (Tables 2.1-2.3). At soil moisture saturation, the cultivar UPLRi5 was more resistant than C22 and Kanandong Patong, whereas on severely drought stressed plants, UPLRi5 was the most susceptible (Table 2.3).

Under field conditions, the effects of drought stress are likely to be even more important on altering the susceptibility of plants to rice blast. During periods of drought, cultivars with short rooting depths and high resistance at soil saturation may be more prone to severe infection than moderately susceptible cultivars which exploit moisture in deeper soil horizons. The time required for plants to attain a stress threshold could increase in cultivars with deep root systems and thus minimize the effects of drought stress on disease development. In plant breeding programs, it may be more efficient to select for partial resistance at three or more stress regimes. In a breeding program, the statistical methodology suggested in this thesis (Chapter 2) could be used to remove the confounding effects of drought stress on leaf age.

In most of my experiments in which relative infection efficiency was scored, there was an apparent stress threshold above which there was a dramatic increase in infections. There are other thresholds in response to drought stress. For example, there was a threshold cumulative stress (MPa days) below which turgid osmotic potential decreased dramatically (Turner et al. 1986). Another threshold was observed by Dingkuhn et al. (1989) in which there was a sudden and rapid increase in stomatal closure and a rapid decrease in photosynthesis at a leaf water potential of -1.0 to -0.7 MPa. Further research is required to determine whether these plant reponses are related to plant susceptibility and to further explain the enviromental impact of the environment on the drought stress threshold for susceptibility.

A few cultivars of rice have been grown in the same area for a number of years, without succumbing to the "boom and bust" cycle (Bonman et al. 1992). Thus, the resistance has been durable. Durable resistance has been associated with partial resistance (Bonman and Mackill 1988). The upland rice cultivars IRAT 13 and IAC 47 were reported to have durable resistance to blast in West Africa and Brazil (Bonman and Mackill 1988). Not all cultivars with partial resistance have been durable, however. In Japan, one cultivar had partial resistance which was under the control of a single gene. This cultivar became susceptible after only one season. In Brazil, after a few cropping seasons, lines with partial resistance were extremely susceptible whereas lines with complete resistance were resistant (Guimaraes et al. 1993). It is possible that drought, which is common in Brazil, plays an important role in the rapid deterioation of partial resistance in these lines.

Pre-inoculation drought stress had little effect on the latent period, lesion size and sporulation capacity (Chapter 3). Thus, infection efficiency was the most important component of the infection cycle that was altered by pre-inoculation drought stress.

Drought stress increased the susceptibility duration of a particular leaf part. This effect of drought stress was related, in part, to differences in the asymptotic relative infection efficiency and rate of infection with leaf wetness duration among leaves (Chapter 4). The effect of drought on both parameters was more important on older than younger leaves, whereas on the expanding leaf, the effect was mainly on asymptotic infection efficiency. An increase in the rate of infection could increase the number of lesions dramatically in the field, even if leaf wetness durations were longer under well watered than dry soil conditions as reported by Bonman et al. (1988). Although it is not appropiate to compare the different experiments in Chapter 4, the results suggested that the effects of drought stress on the leaf age component was more important on older than younger plants. The minimum time required for infection was significantly longer on leaves at lower positions than the expanding leaves. Drought stress significantly decreased this minimum time required for infection.

The effect of leaf age and drought stress could be mediated through a number of factors. In addition to the silica status of the leaf, other mechanisms are possible. The effects of leaf age on the phylloplane microflora should be studied in future research. In strawberry, the phylloplane microflora was an important component in the reduction of infection with leaf age by *Botrytis cinerea* Pers.: Fr. (Braun amd Sutton 1988). The effects of leaf age on the phylloplane microflora on rice is unknown and could be an important attribute to consider when conducting studies on biological control.

It is possible that the effect of drought stress could also be partly mediated through disruption of the epicuticular wax layer by leaf rolling. This could lead to sites of uneven wax distribution on the leaf surface. To understand the mechanism of drought stress on increasing susceptibility further, this possibility should be explored by scanning electron microscopy.

Leaf age related resistance is an important component of partial resistance to blast (Roumen 1993). In fact, there was a strong correlation between the infection efficiencies obtained on different cultivars in the greenhouse and disease severity data collected in the field by Bonman et al. (1988). My simulation study demonstrated that the speed, at which resistance is attained with tissue age, is an important component of partial resistance. In simulations, in which the infection efficiency decreased rapidly with tissue age, disease severity increased dramatically with a small decrease in the rate at which resistance was attained (Fig. 4.2). Increasing the susceptibility duration was as important as increasing the infection efficiency and decreasing the latent period by equivalent amount (Table 4.2, Fig. 4.7).

For this component of partial resistance to be more effective, other modes of resistance are likely required. Although overall disease severity was reduced by shorter susceptibility durations, the disease severity was greater on flushes of growth formed later than early in the season (Fig. 4.3). In rice, this could be important because these later infections are a possible source of inoculum for panicle infections due to the long infectious period and location of the infected leaves. Also, the skewed distribution of lesions toward the top of the canopy would be expected to decrease yields (Waggoner

1990). This situation could be averted by including adult plant resistance (Koh et al. 1988).

The effect of leaf blast on shoot growth as been reported elsewhere (Bastiaans 1993). Shoot growth was reduced (Bastiaans 1993, Chapter 5). However, there are few reports of the effects of blast on root growth. Foliar infections were important in reducing shoot growth. More important, the root growth of drought stressed plants was reduced in the deep soil layers, impeding the ability of plants to avoid drought stress. In addition, proton extrusion was less from roots of inoculated than control plants, with implications for rhizosphere pH and nutrient uptake. These effects could be expected to increase the susceptibility of plants to subsequent infections.

One major way to control blast is through water management. The control of leaf blast in the uplands is difficult, particularly for the poor farmers, because the infrastructure costs for irrigation would be prohibitive. Thus, water conservation methods must be used such that the soil moisture loss is minimized. These methods could include mulching (McLean et al. 1993) to control weeds and to reduce evaporation, and split applications of fertilizer (Kurschner et al. 1992) to reduce crop transpiration by reducing leaf area index. These measures could also have indirect effects on leaf wetness duration, decreasing the speed of disease progress further.

In conclusion, including partial resistance due to differences in leaf susceptibility durations among cultivars, is an effective way to suppress disease progress. The effect of pre-inoculation drought stress on changing this component of infection efficiency was most important; drought stress had little effect on other components.

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